บทบาทของตัวรับซีโรโตนินชนิด 1B/1D และ 2A/2C ในการตอบสนองทางประสาทและหลอดเลือดต่อไนตริกออกไซด์ ในระบบไทรเจมมิโนวาสคูลาร์ ของหนูแรท

นางสาว ชลาลัย สุวัฒนะโสภณ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสรีรวิทยา สหสาขาวิชาสรีรวิทยา
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2544
ISBN 974-03-0617-9

THE ROLE OF 5-$HT_{1B/1D}$ AND 5-$HT_{2A/2C}$ RECEPTORS IN NEUROVASCULAR RESPONSE TO NITRIC OXIDE IN RAT TRIGEMINOVASCULAR SYSTEM

Miss Chalalai Suwattanasophon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Physiology
Inter-department of Physiology
Graduate School
Chulalongkorn University
Academic year 2001
ISBN 974-03-0617-9

Thesis Title	THE ROLE OF 5-$HT_{1B/1D}$ AND 5-$HT_{2A/2C}$ RECEPTORS IN NEUROVASCULAR RESPONSE TO NITRIC OXIDE IN RAT TRIGEMINOVASCULAR SYSTEM.
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ชลาลัย สุวัฒนะโสภณ: บทบาทของตัวรับซีโรโตนินชนิด 1B/1D และ 2A/2C ในการตอบสนองทางประสาทและหลอดเลือดต่อ ไนตริกออกไซด์ ในระบบไทรเจมมิโนวาสคูลาร์ของหนูแรท (THE ROLE OF 5-$HT_{1B/1D}$ AND 5-$HT_{2A/2C}$ RECEPTORS IN NEUROVASCULAR RESPONSE TO NITRIC OXIDE IN RAT TRIGEMINOVASCULAR SYSTEM.) อ. ที่ปรึกษา: รศ. นพ. อนันต์ ศรีเกียรติขจร, อ. ที่ปรึกษาร่วม: รศ. ดร. ปานสิริ พันธุ์สุวรรณ; 161 หน้า ISBN 974-03-0617-9

งานวิจัยนี้มีจุดประสงค์เพื่อศึกษาผลของการกระตุ้นตัวรับซีโรโตนินชนิด 1B/1D และ 2A/2C ต่อกระบวนการรับการกระตุ้นที่รุนแรงของหลอดเลือดสมอง โดยแบ่งหนูพันธุ์วิสต้าเพศผู้ ออกเป็น 4 กลุ่ม ประกอบด้วยกลุ่มที่ได้รับ ซูมาทริปแทน นาราทริปแทน 1,2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) และน้ำเกลือ ทั้งนี้ซูมาทริปแทนและนาราทริปแทนเป็นสารกระตุ้นตัวรับซีโรโตนินชนิด 1B/1D และ DOI เป็นสารกระตุ้นตัวรับซีโรโตนินชนิด 2A/2C ตัวแปรที่ทำการศึกษาประกอบด้วย อัตราการไหลเวียนเลือดเฉพาะที่ของบริเวณเปลือกสมองใหญ่และการแสดงออกของเอนไซม์ไนตริกออกไซด์ซินเทสชนิดนิวโรนาล (nNOS) โดยวัดอัตราการไหลเวียนเฉพะที่ของเปลือกสมองด้วยวิธี laser Doppler flowmetry และศึกษาการปรากฏของเอนไซม์ nNOS บริเวณปมประสาทไทรเจมมินาล กลุ่มเซลล์ไทรเจมมินาลนิวเคลียสคอดาลิสและและใยประสาทรอบหลอดเลือดโดยวิธีอิมมูโนฮิสโตเคมมิสตรี ผลการศึกษาพบว่าทั้งซูมาทริปแทนและนาราทริปแทนไม่เปลี่ยนแปลงอัตราการไหลเวียนเฉพะที่ของเปลือกสมอง ในขณะที่ DOI สามารถเพิ่มการไหลเวียนเฉพะที่ได้อย่างมีนัยสำคัญทางสถิติ งานวิจัยนี้ยังได้ศึกษาผลของการกระตุ้นตัวรับซีโรโตนินชนิด 1B/1D และ 2A/2C ต่อการตอบสนองของระบบไทรเจมมิโนวาสคูลาร์ต่อไนตริกออกไซด์ โดยหยดสารไนโตรกลีเซอรินเข้าหลอดเลือดดำ โดยสารนี้สามารถแตกตัวให้ไนตริกออกไซด์ ผลการศึกษาพบว่าสารไนโตรกลีเซอรินสามารถเพิ่มอัตราการไหลเวียนเลือดเฉพาะที่ของบริเวณเปลือกสมองใหญ่ และฤทธิ์ดังกล่าวคงอยู่เป็นระยะเวลากว่า 60 นาที การศึกษายังพบว่าการกระตุ้นตัวรับซีโรโตนินชนิด 1B/1D สามารถลดผลของไนตริกออกไซด์ในการเพิ่มอัตราการไหลเฉพาะที่ของเลือดบริเวณเปลือกสมองได้ ขณะที่การกระตุ้นตัวรับซีโรโตนินชนิด 2A/2C ไม่มีผลต่อการตอบสนองดังกล่าว ในส่วนของการศึกษาโดยวิธีอิมมูโนฮิสโตเคมมิสตรีพบว่าการให้สารไนโตรกลีเซอรินสามารถเหนี่ยวนำให้เกิดการแสดงออกของเอนไซม์ nNOS ในส่วนต่างๆของระบบไทรเจมมิโนวาสคูลาร์ โดยพบว่าเซลล์ประสาทที่ย้อมติดเอนไซม์ nNOS ในปมประสาทไทรเจมมินาล และกลุ่มเซลล์ไทรเจมมินาลนิวเคลียสคอดาลิสมีสัดส่วนสูงขึ้น และกะเปาะบนใยประสาทรอบหลอดเลือดดำซุปพีเรียเซกจิตาลไซนัสที่ย้อมติดเอนไซม์ nNOS มีขนาดใหญ่ขึ้น การกระตุ้นตัวรับซีโรโตนินชนิด 1B/1D สามารถยับยั้งการตอบสนองของเอนไซม์ nNOS ต่อไนตริกออกไซด์ นอกจากนี้ยังพบว่าการกระตุ้นตัวรับซีโรโตนินชนิด 2A/2C สามารถเหนี่ยวนำให้เกิดการเปลี่ยนแปลงของเอนไซม์ nNOS ในระบบไทรเจมมิโนวาสคูลาร์ในลักษณะเดียวกับการกระตุ้นด้วยไนโตรกลีเซอริน แต่ไม่สามารถเสริมฤทธิ์ของไนโตรกลิเซอรินในการกระตุ้นระบบไทรเจมมิโนวาสคูลาร์ ผลการศึกษานี้บ่งว่าสารไนโตรกลีเซอรินหรือ DOI สามารถกระตุ้นเอนไซม์ nNOS ในระบบไทรเจมมิโนวาสคูลาร์ และทำให้เกิดการสังเคราะห์ไนตริกออกไซด์ขึ้นในร่างกาย ซึ่งส่งผลให้มีการเพิ่มในอัตราการไหลของเลือดบริเวณเปลือกสมองใหญ่เป็นเวลานาน การกระตุ้นตัวรับซีโรโตนินชนิด 1B/1D สามารถลดผลของไนตริกออกไซด์ที่ทำให้เกิดการขยายตัวของหลอดเลือด และการแสดงออกของเอนไซม์ nNOS ในระบบไทรเจมมิโนวาสคูลาร์ได้ ผลการศึกษานี้แสดงให้เห็นถึงบทบาทที่แตกต่างกันของตัวรับซีโรโตนินต่างชนิดในกระบวนการควบคุมการรับการกระตุ้นที่รุนแรงของหลอดเลือดสมอง

หลักสูตร…… ลายมือชื่อนิสิต…….

สาขาวิชา…… ลายมือชื่ออาจารย์ที่ปรึกษา…..……

ปีการศึกษา……. ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……...

4175209530 : MAJOR PHYSIOLOGY
KEYWORD SEROTONIN RECEPTORS/ NITRIC OXIDE/ TRIGEMINOVASCULAR SYSTEM.

CHALALAI SUWATTANASOPHON: THE ROLE OF 5-$HT_{1B/1D}$ AND 5-$HT_{2A/2C}$ RECEPTORS IN NEUROVASCULAR RESPONSE TO NITRIC OXIDE IN RAT TRIGEMINOVASCULAR SYSTEM.THESIS ADVISOR: ASSOC. PROF. ANAN SRIKIATKHACHORN, M.D.THESIS CO-ADVISOR: ASSOC. PROF. PANSIRI PHANSUWAN-PUJITO, Ph.D.161 pp.ISBN 974-03-0617-9

This study was conducted to investigate the effect of two serotonin receptor subtypes, namely 5-$HT_{1B/1D}$ and 5-$HT_{2A/2C}$ receptors on the cerebrovascular nociception. Adult male Wistar rats were divided into four groups, including those pretreated with sumatriptan, naratriptan, 1,2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) and physiologic saline. Sumatriptan or naratriptan were used as 5-$HT_{1B/1D}$ agonist whilst DOI was employed to activate the 5-$HT_{2A/2C}$ receptor. The measured parameters included regional cerebral blood flow (rCBF) and neuronal nitric oxide synthase (nNOS) expression. The change in rCBF was monitored using laser Doppler flowmetry. The degree of nNOS expression was quantitated at the trigeminal ganglion, trigeminal nucleus caudalis and perivascular nerve fiber by immunohistochemical method. The results showed that neither sumatriptan nor naratriptan altered the rCBF. On the other hand, administration of DOI could significantly increase the basal rCBF. To study the effect of these receptor agonists on nitric oxide (NO)-induced change in trigeminovascular system, nitroglycerin was intravenously infused to the animals. The results showed that exposure to NO-donor led to a long-lasting cerebral hyperemia. It was also demonstrated that despite the lack of their efficacy in altering the basal rCBF, pretreatment with 5-$HT_{1B/1D}$ agonists could minimize the degree of NO-induced hyperemia in cerebral cortical tissue. No significant change was observed in the group pretreated with DOI as compared with those receiving nitroglycerin alone. The immunohistochemical study showed that exposure to NO-donating agent could induce expression of nNOS system in various structures of the trigeminovascular pathway. The surface area of nNOS-IR perivascular varicosity around the superior sagittal sinus was greater in the nitroglycerin-treated animals. The number of nNOS-IR neurons in trigeminal ganglion and trigeminal nucleus caudalis were also greater in this group. Pretreatment with 5-$HT_{1B/1D}$ agonist, sumatriptan can attenuate the NO-evoked nNOS expression in all areas. It was also showed that administration of DOI could induce nNOS-IR in these strutures but did not interfere with the effect of NO in the activation of nNOS. Based on these findings, it can be concluded that administration of nitroglycerin or DOI results in long-lasting vasodilation by activating the endogenous nNOS system. The effect of NO in induction of vasodilatation and nNOS expression can be attenuated by prior activation of 5-$HT_{1B/1D}$ receptor. These results indicate that different serotonin receptors exert different role in control of cerebrovascular nociception.

Department……..…… Student's signature……..……

Field of study…….. Advisor's signature……..……

Academic year…… Co-advisor's signature……..……..

ACKNOWLEDGEMENTS

I wish to express my gratitude and appreciation to my kind advisor, Associate Professor Dr. Anan Srikiatkhachorn MD, for his excellent instruction, guidance, encouragement and support during the long working process, which enable me to carry out this study. His kindness will be long remembered.

I would like to express my gratitude to Associate Professor Dr. Pansiri Phansuwan for training me about immunohistochemical technique and helping me to more deeply understand my research. Her tireless and dedicated devotion to my thesis will be in my mind forever.

I would like to thank Associate Professor Dr. Suthiluk Patumraj for training me about laser doppler flowmeter technique.

I wish to give a very special vote to thank to Miss Supang Maneesri, Miss Juntima Pattamanont and Miss Pilaiporn Thongsaiporn for their assistance, cheerfulness and support my mind throughout this study. My thanks also go to Dr. Amporn Jariyapongskul for training me about cranial window technique.

I wish to express my sincere thanks to all my teachers and friends at Department of Physiology, Faculty of Medicine, Chulalongkorn University who are not mentioned here for all their loving helps during the time I was studying. My special thanks are also extended to my former teachers who have giving me the knowledge and scientific attitude.

My thanks also go to the Department of Anatomy, Faculty of Medicine, Srinakarinwirot University for the use of their facilities.

My appreciation is also devoted to my dear father, sister, aunt and Mr. Piyaphan nuttapho for their love, kindness and understanding.

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LISE OF ABBREVIATIONS

μm = micrometer
5-HIAA = 5-Hydroxyindoleacetic acid
5-HT = serotonin
ABC = avidin biotin complex
B.W. = body weight
BSA = bovine serum albumin
C_1 = cervical spinal cord segment 1
C_2 = cervical spinal cord segment 2
Ca^{2+} = calcium ion
CCK = cholecystokinin
CGRP = calcitonin gene-related peptide
DAB = 3,3-diaminobenzidin
DOI = 1,2,5-dimethoxy-4-iodophenyl-2- aminopropane
K^+ = potassium ion
kg = kilogram
mCPP = m-Cholorophenylpiperazine
mg = milligram
mm = millimeter
NMDA = N-methyl-D- aspartate
nNOS = neuronal nitric oxide synthase
nNOS-IR = neuronal nitric oxide synthase-immunoreactive
NO = nitric oxide
NOS = nitric oxide synthase
NSS = normal saline
NTG = nitroglycerin
PBS = phosphate buffer saline
rCBF = regional cerebral blood flow

SP = substance P

TNC = trigeminal nucleus caudalis

VIP = vasoactive intestinal polypeptide

CHAPTER I
INTRODUCTION

Migraine is a syndrome that affect a substantial fraction of world's population, with a higher prevalence in females (15-18 %) than in males (6 %; Lipton and Stewart, 1997). Migraine is characterised by attacks of intense, pulsatile and throbbing headache, which is typically unilateral and is accompanied by anorexia, nausea, vomiting and photo- and/or phonophobia. In the present, It can proof that the perivascular (trigeminal) sensory nerve activity provokes headache and other associated symptoms. This stimulation of trigeminal nerve may also release neuropeptides and nitric oxide, thus reinforcing vasodilatation and perivascular sensory nerve activity.

Theoretically, nitric oxide may be implicated in the pathophysiology of migraine. Thus, endothelium-dependent vasodilatation is of importance in cerebrovascular regulation, in addition neurogenic vasodilatation may be mediated via perivascular nerve, which operate through NO (nonadrenergic, noncholinergic nerves). Furthermore, NO mediated neurotransmission in the central nervous system, is important for pain perception (hyperalgesia). NO may also contribute to sensory transmission in peripheral nerves. Finally, NO may release CGRP from perivascular nerve ending and may thus play a role in neurogenic inflammatory reactions.

Nitroglycerin (NTG) is substance that reliably and dose dependently produce headache in normal volunteers and migraine sufferes (Olesen et al., 1993). NTG itself has no know action in the human body but acts to liberate NO and is thus generally regarded as an NO donor (Ignarro et al.,

1981). Previous studies have suggested that migraine patients experience a migraine-like headache in association with NTG administration more often than do non-migraineurs (Sicuteri, 1987). Thus, migraineurs are hypersensitive to nitroglycerin-induced headache and most likely therefore to nitric oxide. An increased headache response could, however, reflect a greater general sensitivity to pain, or it could be due to increased physiological sensitivity to NO. It is well known that nitroglycerin dilates the middle cerebral artery via NO without affecting cerebral blood flow and thereby the arterioles (Dahl et al, 1989; Iversen et al 1989a).

Several results indicate that serotonin (5-HT) play an important role in the pathogenesis of migraine (Raskin, 1991). Urinary excretion of 5-hydroxyindole-3-acetic acid, the metabolite of 5-HT, has been reported to increase during the headache phase (Sicuteri et al, 1961). Whereas platelet 5-HT levels decrease (Anthony et al., 1969). Intravenous administration of 5-HT can alleviate migraine symptoms elicited by 5-HT depleting agents (Kimball et al., 1960). Recently, $5\text{-HT}_{1B/1D}$ receptor agonist was used for migraine treatment because it can reduce the neuropeptide release which cause of vasoconstriction and decrease neuronal activity of trigeminal nerve. On the other hand, The activation of 5-HT_{2C} receptor might provoke the sterile inflammatory response on the cerebral vasculature because of the release of NO (Glusa and Richter, 1993). Therefore, the activation of this recepter might be a key step in the development of migraine (Fozard and Kalkman, 1994).

As previously mentioned, NO and 5-HT are involved in pathophysiology of migraine but the relation of NO and 5-HT is unclear. Therefore, we design to study the effect of each 5-HT receptor agonist to NO on trigeminovascular system.

CHAPTER II
REVIEW LITERATURE

TRIGEMINOVASCULAR SYSTEM

The trigeminal nerve is the largest cranial nerve and contains both sensory and motor fibers. It is the sensory nerve to the greater part of the head and the motor nerve to several muscles, including the muscles of mastication. The trigeminal nerve has three branches, the ophthalmic, maxillary and mandibular divisions (Snell, 1992).

The dura and cranial blood vessel, which are pain sensitive structures, are also supplied by trigeminal afferents, particularly from the ophthalmic and mandibular divisions (Arbab et al., 1986; Andres et al., 1987). Surrounding the large, supratentorial cerebral vessels, pial vessels, large venous sinuses, and dura mater is a plexus of nociceptive fibers, largely unmyelinated (Aδ) fibers and unmyelinated (C) fibers, that arise from the trigeminal ganglion, while the posterior fossa innervation arises from the upper cervical dorsal roots (Goadsby, 1997). The cell bodies of most of the afferent fibers lie in the trigeminal (Semilunar or Gasserian) ganglion in the middle cranial fossa at the base of the skull (Waite and Tracey, 1995). Trigeminal ganglion cells are pseudounipolar and can also be classified on the basis of ultrastructural and immunocytochemical differences (Kai-kai, 1989), into large, type A cells and smaller, type B cells, with subclasses of each trigeminal ganglia cells also contain amino acids and neuropeptides similar to those in spinal ganglia (Kai-Kai, 1989; Ichikawa et al., 1993; Liu et al., 1993). In general, dorsal root ganglion cells contain one or more peptides. Some of the peptides that have been

identified in dorsal root ganglion cells by immunohistochemical staining include the following: substance P (SP), somatostatin (SOM), cholecystokinin (CCK), calcitoningene-related peptide (CGRP), bombesin, vasoactive intestinal polypeptide (VIP), galanin, vasopressin, oxytocin, dynorphin (DYN), enkephalin (ENK), α-neoendorphin, corticotropin-releasing factor, and neurokinin A (Willis and Coggeshall, 1991). To date it is unclear if the presence of a particular set of peptides can predict the function type of sensory receptor (Cameron et al., 1988). It seems likely that the peptides are neuromodulators that act in concert with fast-acting neurotransmitters, either enhancing or diminishing their action (Willis et al, 1995).

Moreover, tracing studies have shown that fibers innervating cerebral vessel arise from the trigeminal ganglion in neurons that contain SP and CGRP, both of which can be released when the trigeminal ganglion is stimulated (Goadsby and Edvinson, 1994). Recently, Hou and his coworker were reported the expression of 5-HT_{1B} and 5-HT_{1D} receptor colocalize with CGRP, SP and nitric oxide synthase (NOS) (Hou et al., 2001). Neuropeptides that released by antidromic activation of the trigeminal nerve such as CGRP, SP and neurokinin A, induce vasodilatation (Moskowitz, 1993).

The trigeminal sensory nuclei extend from the midbrain to the upper cervical spinal cord and are divided into three main groups: the mesencephalic nucleus, the main or principal sensory nucleus, and the spinal trigeminal nucleus. Spinal trigeminal nucleus is itself subdivided into oral, interpolar and caudal parts. About half the trigeminal fiber divide into ascending and descending branches when they enter the pons; the remainder ascend or descend with out division. The ascending branches

terminate in the main sensory nucleus and the descending branches terminate in the spinal nucleus. The sensation of touch and pressure are conveyed by nerve fibers that terminate in the main sensory nucleus. The sensations of pain and temperature pass to the spinal nucleus. The sensory fibers from ophthalmic division of the trigeminal nerve terminate in the inferior part of the spinal nucleus (caudal part); fibers from the maxillary division terminate in the middle of spinal nucleus (interpolar part); and fibers from the mandibular division end in the superior part of the spinal nucleus (oral part) (Snell, 1992).

Trigeminal nucleus caudalis (TNC) extends from the level of the obex to the upper cervical dorsal horn with which it is continuous. TNC, which receive nociceptive afferent fibers from the ophthalmic division of trigeminal nerve, has functional and anatomical organization of nociceptive neurons as same as the dorsal horn of the spinal cord. Nociceptive neurons are located in the superficial dorsal horn, in the marginal layer (also called lamina I) and the substantial gelatinosa (lamina II). The majority of these neurons receive direct synaptic input from Aδ and C fibers. Many of the neurons in the marginal layer (lamina I) respond exclusively to noxious stimulation (and thus are called nociceptive-specific neurons) and project to higher brain centers. Some neurons in this layer, called wide-dynamic-range neurons respond in a graded fashion to both nonnoxious and noxious mechanical stimulation. The substantial gelatinosa (lamina II) is made up almost exclusively of interneurons (both excitatory and inhibitory), some of which respond only to nociceptive inputs while other respond also to nonnoxious stimuli. Lamina III and IV contain neurons that receive monosynaptic input from Aβ fibers. Lamina V contains primarily wide dynamic-range neurons that project to the brain stem and to region of the thalamus. These neurons receive monosynaptic input from Aβ and Aδ

fibers. They also receive input from C fibers, either directly on their dendrites, which extend dorsally into the superficial dorsal horn, or indirectly via excitatory interneurons that themselves receive input directly from C fibers (Figure 2.1)

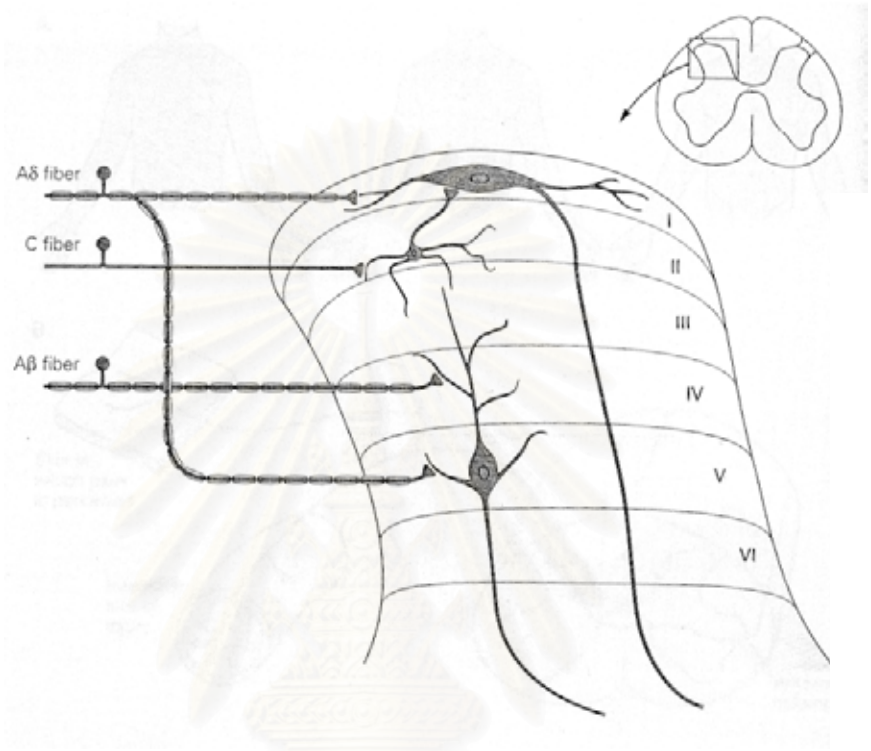


Figure 2.1. *Nociceptive afferent fibers terminate on projection neurons in the dorsal horn of the spinal cord. Projection neurons in lamina I receive direct input from myelinated (Aδ) nociceptive afferent fibers and indirect input from unmyelinated (C) nociceptive afferent fibers via stalk cell interneurons in lamina II. Lamina V neurons are predominately of the wide dynamic-range type. They receive low threshold input from the large-diameter myelinated fibers (Aβ) of mechanoreceptors as well as both direct and indirect input from nociceptive afferent fibers (Aδ and C). In this figure the lamina V neuron sends a dendrite up through lamina IV, where it is contacted by the terminal of an Aβ primary afferent. A dendrite in lamina III arising from a cell in lamina V is contacted by the axon terminal of a lamina II interneuron (Basbaum and Jessel, 2000).*

Cells cotaining GABA, glutamate or aspartate are seen in all layers (Haring et al., 1990; Magnusson et al., 1986). Colocalization of glutamate with SP and CGRP has been demonstrated in primary afferent terminal in the dorsal horn (Wiesenfeld-Hallin et al., 1984). The second order neurons that lie in the TNC and in the dorsal horn of upper cervical spinal cord at the C_1 and C_2 levels project their fibers via quintothalamic tract. This tract decussates before synaping on third order neurons in the thalamus (Goadsby, 1997). Traditionally TNC and its thalamic projections have been considered to be the pathway responsible for temperature sensibility and nociception from cranial inputs, analogous to the dorsal horn and spinothalamic pathway in the spinal cord.

Furthermore, second order neurons of TNC synapses in the superior salivatory nucleus, which is parasympathetic outflow (Goadsby, 1997). The parasympathetic innervation of the head arises from cell bodies in the superior salivatory nucleus, passes out with fibers of the facial and glossopharyngeal nerve; and synapses in the sphenopalatine and otic ganglia before reaching the vessels. The classical transmitters in these systems are noradrenaline and acetylcholine. During the part 25 years it has been demonstrated that some perivascular autonomic nerves contain other so called NANC (non-adrenergic non-cholinergic) transmitters or modulator substances. In the parasympathetic system, peptide histidine methionin (PHM) NO are transmitters or modulators (Thomsen, 1997; Nozaki, 1993). Most of the neurovascular nerve fiber display neuronal nitric oxide synthase (nNOS) immunoreactivity, which produce NO, justifying the novel term “ nitroxergic nerves”. Berger et al has revealed the presence of nNOS in nerve fibers of the rat dura mater (Berger et al., 1994). Being a potent vasodilator, NO was proposed to participate in the

pathogenesis of vascular headache by activation of nitroxergic nerves in a manner similar to CGRP (Goadsby and Evinsson, 1993).

BRAIN STEM PROCESSING OF TRIGEMINOVASCULAR PAIN

The sites within the brain stem that are responsible for craniovascular pain now have begun to be mapped. Using c-*fos* immunocytochemistry a method for looking at activated cells- after meningeal irritation with blood or administered intracisternaly of capsaicin, expression was reported in the TNC (Nozzaki et al., 1992; Mitsikostas et al., 1998). Moreover, an increase in c-*fos* expression was observed in the TNC and the probability of firing, using electrophysiological methods, was recorded in the neurons of dorsal C_2 spinal cord (TNC) after superior sagittal sinus stimulation (Kaube et al, 1993a; Strassman et al., 1994; Goadsby and Hoskin, 1998). In these experimental models, mechanical, chemical or electrical activation of the C and Aδ fibers of the trigeminal nerve induce c-*fos* expression within specific areas of the TNC via release of neurotransmitters from the central terminal (Bereiter and Benetti, 1996). From TNC, pain is conducted to other brainstem nuclei and to higher cortical structures for registration and modulation of nociceptive information.

THE LINK BETWEEN GLUTAMATE AND NITRIC OXIDE

Nociceptive afferent fiber use glutamate and neuropeptides as neurotransmitters. Synaptic transmission between nociceptors and dorsal horn neurons is mediated by chemical neurotransmitter released from central sensory nerve endings. The primary afferent fibers of nociceptive neurons also elicit slow excitatory postsynaptic potential in dorsal horn

neurons by releasing peptide transmitters. Small-diameter primary afferent terminals in the dorsal horn contain both small electron-translucent synaptic vesicles that store glutamate and large dense-core vesicles that store neuropeptides. Glutamate and neuropeptides are released together from primary afferent terminals and have distinct physiological actions on postsynaptic neurons, but they act coordinately to regulate the firing properties of postsynaptic neurons. Neuropeptides, including substance P, appear to enhance and prolong the actions of glutamate. In the spinal cord, glutamate is neurotransmitter that release from nociceptive afferent fibers at the central end. Glutamate is confined to postsynaptic neurons, which have glutamate receptor. The acute action of glutamate at NMDA receptor activation may also be involved in the hyperalgesia, synaptic plasticity. In the spinal cord, hyperalgesia is produced by high-frequency C-fiber strength electrical stimulation (Woolf, 1989). In contrast, low-frequency or A-fiber strength stimulation will not produce hyperalgesia (Woolf, 1992). Therefore, it has been proposed (Woolf, 1989, 1992; Dubner and Ruda, 1992) that high-frequency barrages of C-fiber discharges after injury result in release of glutamate and neurokinins (and likely other substances) from primary afferent terminals, fast synaptic potentials produced by action at non-NMDA excitatory amino acid receptors (i.e, AMPA/KA), and slow synaptic potentials produced by continual depolarization and peptides (i.e. SP, CGRP) (Meller and Gebhart, 1993). The NMDA receptor remains inactive under physiological concentrations of Mg^{2+} and even in the presence of released glutamate. The expulsion of Mg^{2+} from the ion channel allows for an influx of Ca^{2+} through the NMDA receptor ion channel. This leads to a sequence of events that probably include activation of a wide range of protein kinases, production of NO and activation of immediate early genes to produce the long-term changes found in hyperalgesia and LTP (Meller and Gebhart, 1993).

In addition to many of the NMDA receptor is a voltage-gated ion channel that, once activated, allows Ca^{2+} to enter the neuron. This increase in intracellular Ca^{2+} triggers a cascade of events that include stimulation of phospholipases to produce diacylglycerol (DAG) and ecosanoids, stimulation of the production of inositol 1,4,5-triphosphate (IP_3), activation of protein kinase C (PKC) and, importantly, activation of the constitutive form of nitric oxide synthase (NOS) (Figure 2.2). Recently, NO play a role in synaptic transmission in both the central and peripheral nervous systems, have been implicated in nociception and pain processing (Meller and Gebhart, 1993).

CHEMISTRY OF NITRIC OXIDE (NO)

The biochemistry of nitrogen monoxides involves an array of interrelated redox forms. Under physiological conditions there is interconversion among the different redox form (Stamler et al., 1992a). The redox form normally referred to as nitric oxide, or simply NO, is the highly reactive free radical $NO^{\bullet}$ (NO dot), an inorganic gas of formula $^{\bullet}N=O$ (Kiechle and Malinski, 1993). In this neural redox form, NO is poorly soluble in water and easily penetrates biological membranes (Kiechle and Malinski, 1993). The half-life of NO is reported to be the range 5-30 sec under bioassay condition (Palmer et al., 1987; Kiechle and Malinski, 1993). Under physiological conditions (i.e. in an oxygenated and heated aqueous solution with pH around 7.4) NO is rapidly converted to nitrogen dioxide (NO_2), which again rapidly forms the more stable metabolites nitrite (NO_2^-) and nitrate (NO_3^-) (Wennmalm and Peterson, 1991). NO also readily reacts with superoxide (O_2^-) to form the unstable and toxic metabolite

peroxynitrite ($ONOO^-$). The effects of NO are, therefore, prolonged when O_2^- is degraded by superoxide dismutase (SOD) (Gryglewski et al., 1986).

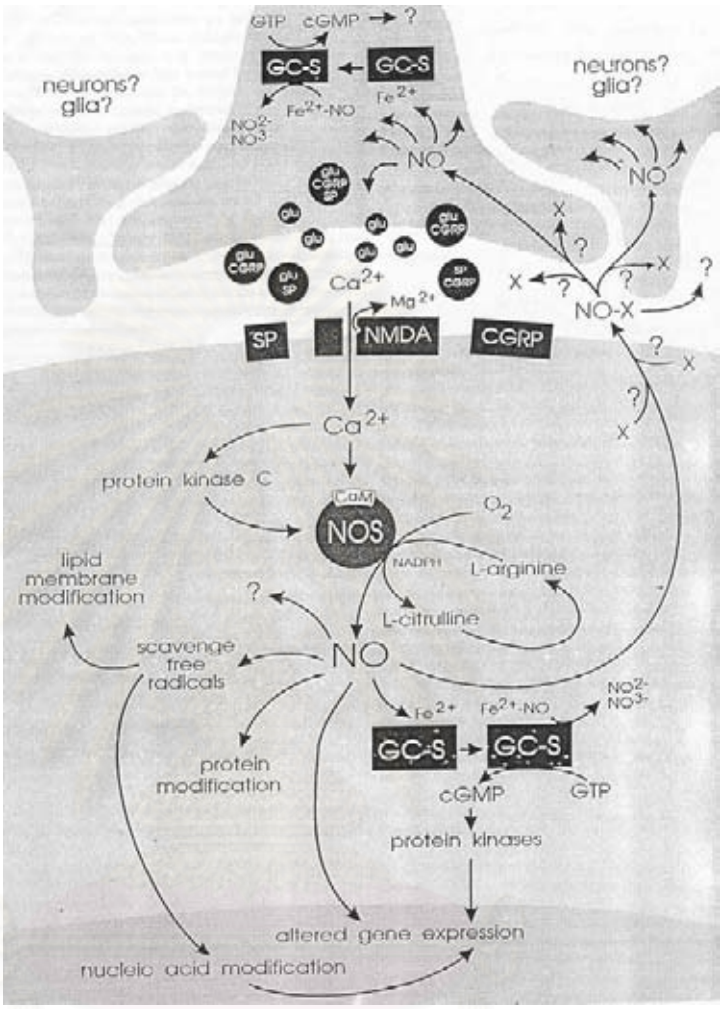


Figure 2.2 *Summary diagram illustrating many of the interactive mechanisms that may ensure as a result of NMDA receptor activation and NO production. Refer to the text for details of the actions depicted in this figure. Although not diagramatically illustrated, all of the interactions that are represented in the post-synaptic neuron may also occur either in the presynaptic neuron or adjacent glia or neurons. Abbreviations CaM = calmodulin, CGRP = calcitonin gene-related peptide, GC-S = soluble guanylate cyclase, glu = glutamate, NO = nitric oxide, NOS = nitric oxide synthase, SP = substance P (Meller and Gebhart, 1993).*

NITRIC OXIDE SYNTHASE (NOS)

In biological systems, NO is generated from the terminal guanidino of L-arginine. This oxidative reaction requires NADPH, O_2, flavins (FMN and FAD) and tetrahydrobiopterin (BH_4) and yields citrulline as a coproduct (Mayer, 1994) (Figure 2.3). The transport of L-arginine within cells involves cationic amino acid transport systems (Schmidt et al., 1993; Greene et al., 1993). Under normal physiological conditions the supply of L-arginine seems not to be the rate-limiting step (Mayer, 1994; Hecker et al., 1990). The enzymes responsible for NO synthesis are known as NO synthase (NOS). NOS activity has been reported in many tissues, including endothelium, brain, peripheral nerves, vascular smooth muscle, myocardium, macrophages, neutrophils and microglia of several spicies (Kiechle and Malinski, 1993; Knowles and Moncada, 1994). Purification and cloning of NOS gene has revealed the existence of at least three isoforms of NOS. Two of these are constitutive, Ca^{2+}/ calmodulin-dependent and release NO from for example endothelium (eNOS) and neurons (nNOS). Another NO synthase (iNOS) is inducible and generally Ca^{2+} independent (iNOS activity which is Ca^{2+} dependent, however, has been reported recently). After induction of iNOS, NO is released from macrophages, astrocytes, microglia and vascular smooth muscle cells for long periods and in large amounts in response to for example endotoxins and cytokines (Busse and MÜlsch, 1990; Stuehr et al., 1991; Wallace and Bisland, 1994).

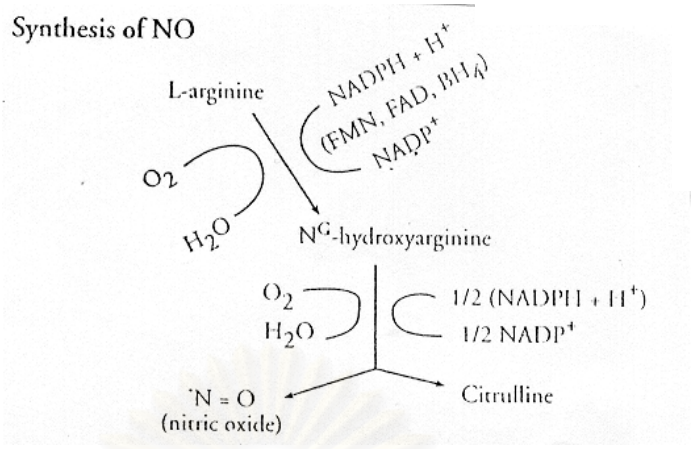


Figure 2.3 *Synthesis of NO from L-arginine. This two-step oxidation requires NADPH, O_2 flavins (FMN and FAD) and tetrahydrobiopterin (BH_4) as co-factors and yields citrulline as a co-product.(Thomsen, 1997).*

EVIDENCE FOR A ROLE OF NITRIC OXIDE IN THE SPINAL CORD

Given that there appears to be a considerable amount of evidence to implicate NMDA in hyperalgesia in the spinal cord, and as many of the effects of NMDA receptor activation appear to be ultimately mediated through production of NO. It is an obvious step to propose that NO play a significant role in hyperalgesia in the spinal cord. However, to date there is little evidence in support and it is not clear how NO may function or exactly what role it may play in the spinal cord.

There is evidence to suggest that NO may be produced by dorsal root ganglion neurons maintained in culture (Bauer et al., 1992) and that NOS (Meller, Bauer, Simmons, Murphy and Gebhart, unpublished observations) and NADPH-diaphorase-labeled neurons (Aimi et al., 1991; Morris et al., 1992; Traub et al., 1992) have been localized in neonatal and adult dorsal root ganglia *in vivo* and *in vitro.* This suggests that NO may be produced

and released in dorsal root ganglia, as well as at the peripheral and central terminals of these neurons. Morris et al. have suggested that NO produced in neurons in dorsal root ganglia may act as a signaling system between neurons and satellite cells (glia) in sensory ganglia (Morris et al., 1992). However, there also is evidence of a potential role for NO at peripheral and central terminals of these neurons. In the periphery, administration of alternate substrates of NOS (antagonists) such as N^G-nitro-L-arginine methyl ester (L-NAME) or N^G-monomethyl-L-arginine (L-NMMA) block the edema associated with intraplantar injection of bradykinin, phospholipase A_2 or P (Hughes et al., 1990; Cirino et al., 1991; Ialenti et al., 1992) and activation of the production of NO has been shown to produce antinociception (Duarte et al., 1990; Meller et al., 1990; Moore et al., 1991). At the central ends of these afferents, recent evidence has shown that there are NADPH-diaphorase and NOS-positive terminal located in the superficial layers of the dorsal horn (Valtschanoff et al., 1992). Therefore, if NO able to be produced in the central terminals of primary afferents, it might function as a classical transmitter in the spinal cord. Evidence from the laboratory of Meller et al.(Meller et al., 1992a) suggests that NO may not be a transmitter at primary afferents because antagonists of NOS do not alter baseline nociceptive reflexes. They do, however, abolish facilitation of nociceptive reflexes (Meller et al., 1992a,b) and it is thus likely that NO, produced in neurons in the spinal cord that contain NOS, like NMDA, plays a role in multi synaptic nociceptive processing in the spinal cord. There have, however, only been a few recent studies that have examined a potential role for NO in spinal nociceptive processing (Haley et al., 1992; Kitto et al., 1992; Meller et al., 1992a,b).

Intrathecal administration of NMDA produces a rapid, transient, dose-dependent thermal hyperalgesia. This hyperalgesia is reversible

blocked by prior treatment with the selective NMDA receptor antagonist, AP5, suggesting that it was due to NMDA receptor activation (Kolhekar et al., 1992; Meller et al., 1992a). Importantly, the hyperalgesia was also reversibly blocked by prior administration of L-NAME (Kitto et al., 1992; Meller et al., 1992a) or methylene blue (an inhibitor of GC-S) (Meller et al., 1992a). In support of previous findings, the dose of AP5 used did not significantly alter baseline tail-flick latency; L-NAME and methylene blue also did not affect baseline tail-flick latency. These results suggest that NMDA produces acute thermal hyperalgesia is reversibly blocked by administration of hemoglobin (Kitto et al., 1992), suggesting that once NO is produced it must leave the neuron where it is produced and travel extracellularly to another neuron (presynaptically) or glia to activate GC-S and subsequently increase production of cGMP. If NO mediates these events, it would be expected that administration of L-arginine, which would increase the production of endogenous NO, might also produce a facillitation of the nociceptive tail-flick reflex with a similar time course and magnitude as NMDA. In support of this, we found that intrathecal administration of L-arginine, but not D-arginine also produced a rapid, transient, dose-dependent thermal hyperalgesia of similar magnitude and time course to that produced by NMDA (Meller et al., 1992a).

NITRIC OXIDE IS A KEY MOLECULE IN HEADACHE

On the basis of animal experiments, it has been suggested that migraine pain is due to perivascular neurogenic inflammation around dural and meningeal arteries. This process is known to be associated with liberation of neuropeptide transmitters from perivascular trigeminal nerve ending (Moskowitz and Macfarlance, 1993). This reaction involves the liberation of several neuropeptides, primarily substance P and

calcitoningene-related peptide (CGRP) (Buzzi and Moskowitz, 1990). Unfortunately, neurogenic inflammation has never been shown to occur around cranial blood vessels during migraine attacks and the concentration of substance P in external and internal jugular venous blood remains normal during migraine attacks (Goadsby et al., 1990). Furthermore, the endothelin-receptor antagonist, Bosentan, and the substance P-antagonist, RPR 100893-201, both block neurogenic inflammation but have no effect in aborting migraine attacks (May et al., 1996). Also NPY and VIP, which are important neuropeptides found in sympathetic and parasympathetic nerve fibers around the intra- and extracranial blood vessels, remain normal in blood from the internal and external jugular veins during migraine attacks (Goadsby et al., 1990). The only peptide known to be released during migraine attacks is CGRP. The concentration of this peptide is increased in the external but not in the internal jugular venous blood (Goadsby et al., 1990). However, CGRP does not cause pain either infused intravenously or injected into the superficial temporal muscle (Pedersen-Bjerregard et al., 1991). It does not even potentiate pain induced by other substances (Pedersen-Bjerregard et al., 1991). The spectacular therapeutic effect of 5-HT receptor agonists in migraine might indicate that 5-HT is involved. However, 5-HT inject intravenously or locally into the superficial temporal muscle does not cause pain (Jensen et al., 1990; Robert, 1992) and it only slightly aggravates bradykinin-induced pain in the temporal muscle (Jensen et al., 1990). It may be concluded that none of the above-mentioned peptides or monoamines are likely to cause the nociception responsible for migraine pain (Thomson and Olesen, 1998).

Nitric oxide has an amazing number of physiologic effects throughout the body of which several, theoretically, may be implicated in the pathophysiology of migraine. Thus, endothelium-dependent vasodilatation is of importance in cerebrovascular regulation, and, in

addition, neurogenic vasodilation may be mediated via perivascular nerves, which operate through NO (non-adrenergic non-cholinergic NANC nerves). Furthermore, NO mediates neurotransmission in the central nervous system of importance for pain perception (hyperalgesia). NO may also contribute to sensory transmission in peripheral nerves. Moreover, NO contributes to the control of platelets and, when produced in large amounts, NO contributes to host defence reactions of importance in non-specific immunity and neurotoxicity (Moncada et al., 1991). Finally, NO may release CGRP from perivascular nerve ending (Wei et al., 1992). Indeed increasing scientific evidence suggests a key role of NO in migraine. There are several evidence support that headache induced by i.v. infusion of nitroglycerin (NTG) (exogenous NO donor) and histamine which liberates NO from vascular endothelium. Thomsen and Olesen suggest that nitric oxide NO is a more likely candidate molecule. The present review deals with the biology of this small messenger molecule and the scientific evidence suggesting a key role for this molecule in migraine headache (Thomsen and Olesen, 1998).

NITRIC OXIDE DONOR INDUCED HEADACHE

One way of testing whether a given molecule is involved in a suspected process is by means of provocation with the substance. The short half-life of NO, and the potential toxicity when produced or given in excess, makes it difficult to administer NO directly in clinical studies. Instead, administration of a NO donor may provide some answers. The nitro-vasodilators are a diverse group of pharmacological agents which produce vascular relaxation by releasing NO and, therefor, activating sGC (Axelsson et al., 1979; Feelisch and Noack, 1987; Gruetter et al., 1981). The mechanisms by which these compounds release NO vary. Sodium-

nitroprusside, nitrosamines and nitrosothiols release NO non-enzymatically, whereas organic nitrates release NO via an enzymatic process which is not fully understood (Ignarro, 1989; Feelisch, 1993; Harrison and Bates, 1993). *In vivo*, organic nitrate esters may interact with free thiols such as cysteine in cells to generate labile intermediate s-nitrosothiols which spontaneously liberate NO (Ignarro, 1989; Harrison and Bates, 1993). This group of compounds includes NTG, which has been used safely in the treatment of angina pectoris for more than a century.

NTG itself has no known action in the human body but acts via liberation of NO and is thus generally regarded as a NO donor (Ignarro et al., 1981; Gruetter et al., 1981; Feelisch and Noack, 1987). NTG is the most suitable substance for experimental studies of NO-induced headache since it is well tolerated and diffuses freely across membranes due to its lipid solubility. It may thus deliver NO to several tissues including those protected by the blood brain barrier (Iversen et al., 1992) validated the headache-inducing properties of this compound in non-migraineurs and developed a reproducible experimental headache model based on intravenous infusion of NTG and recordings of headache characteristics and intensity. The latter on a 0 to 10 point verbal rating scale. The NTG-induced headache in non-migraineurs was found to be of mild or moderate intensity. Steady state was reached after approximately 10 min of infusion. The headache rapidly disappeared after the NTG infusion was stopped. Headache responses were dose-dependent up to 0.5 μg/kg/min, after which a ceiling effect was observed (Iversen et al., 1992). The headache in non-migraineurs had some of the features of a migraine attack (as defined by the diagnostic criteria of the of the IHS) (Headache Classification Committee, 1998), but differed from migraine by being milder and without nausea, photo- and phonophobia (Iversen and Thomsen, 1995).

Interestingly, the NO-donating properties of NTG seem to be important for the induced headache. Thus, N-acetylcysteine, which augments physiological effects of NTG probably by donating SH groups, also augments the headache response to NTG and prolongs extracranial arterial dilatation (Iversen, 1992). Furthermore, NTG-induced headache in normal controls is short lived and is therefore unlikely to be caused by metabolites other than NO, since these have a longer half-life. Finally, the long-acting nitrate 5-isosorbide mononitrate has a long half-life and apart from NO it has other metabolites than NTG. It causes a long-lasting headache and long-lasting arterial dilatation in dose-dependent fashion (Iversen et al., 1992).

In animal experiment, NTG infusion can induce c-fos expression in several area of brain including lamina I, II of TNC which is nociceptive input of animal head (Tassorelli et al., 1999). In addition, NTG applied locally to the pial surface induces release of CGRP from perivascular nerve fibers (Wei et al., 1992) and hence the observed increase in concentration of CGRP during migraine attacks may be secondary to NO formation (Olesen et al., 1994)

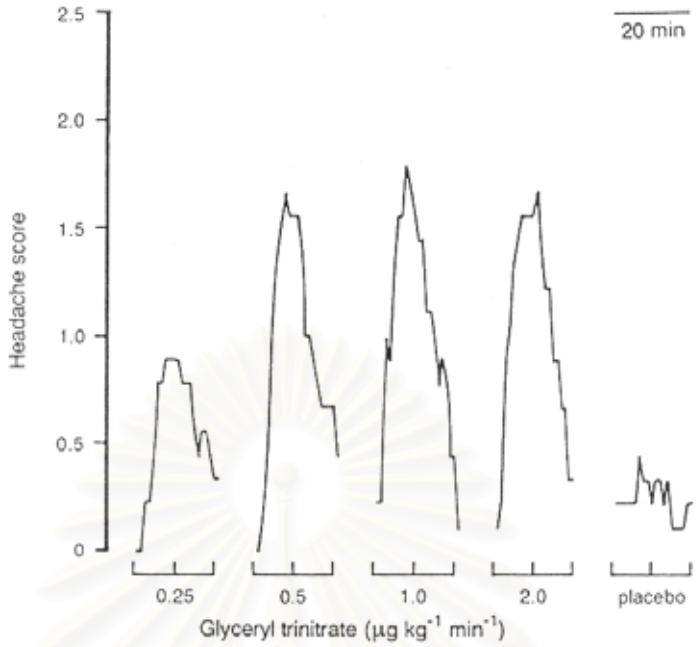


Figure 2.4 *Mean headache scores (0-10 scale) during and after four doses of intravenous glyceryl trinitrate in normal headache-free subjects on day 2 of two separate study days. Glyceryl trinitrate was infused for ten minutes, and during this period a rapid increase in headache was observed. This was followed by a ten-minute wash-out period, which resulted in a rapid decrease in headache. There was a relatively low day-to-day variation and a ceiling effect at approximately 0.5 µg/kg/min. Reproduced, with permission (Iversen et al., 1992).*

Histamine is another compound, which has been known for ages to cause headache (Clark et al., 1936; Northfield, 1938). Activation of endothelial histamine H_1 receptors induces the formation of endogenous NO (Toda, 1990; Fujiwara et al., 1992). Since headache induced by histamine is blocked almost completely by the histamine H_1-blocker mepyramine, but only to a lesser degree by the H_2 blocker cimetidine (Krabbe and Olesen, 1980). NO may also be involved in histamine-induced headache. NTG may induce liberation of histamine from mast cells and

basophils (Rozniecki et al., 1989). However, whereas mepyramine blocked the immediate histamine response (Lassen et al., 1995) it did not prevent NTG-induced immediate headache (Iversen and Olesen, 1994). Thus, a likely final common mechanism of histamine induced and NTG-induced immediate headache is not explained by histamine release after NTG infusion.

More direct evidence for the causal relationship between histamine-induced headache and H_1 receptor mediated activation of the NO pathway would be provided if inhibition of NOS proved to have any effect on histamine-induced headache. A recent study, however, did not show any effect of the competitive NOS inhibitor N-monomethyl-L-argenin (L-NMMA) on histamine-induced headache in healthy subjects (Schmetterer et al., 1997). One explanation for this may be that the administered doses of L-NMMA were too low to outbalance the administered histamine doses.

The formation of NO in a cerebral artery is shown in Figure 2.5. A possible mechanism whereby NO could cause headache is by dilatation of cerebral and extracerebral blood vessels, and this is supported by studies in which arterial dilatation during and after infusion of NTG was consistently observed (Dahl et al., 1989; Thomsen et al., 1993). However, this may not be the only or even the most important mechanism since in healthy subjects the dilatation persists longer than the headache. Furthermore, moderate mechanical dilatation may not cause pain even though vigorous dilatation in association with balloon angioplasty does induce severe headache. Another possibility is that NO cause headache via a direct effect on perivascular sensory nerves. However, cerebral neurons that do not contain NO is present in cerebral periarterial nerves, and predominantly in those arising from the sphenopalatine ganglion (Nozaki et

al., 1993). Few if any of the fibers projecting to the trigeminal ganglion contain NOS are particularly vulnerable to the toxic effects of NO, and since NO is a noxious molecule (Dawson et al., 1992), it might activate sensory nerve fibers directly.

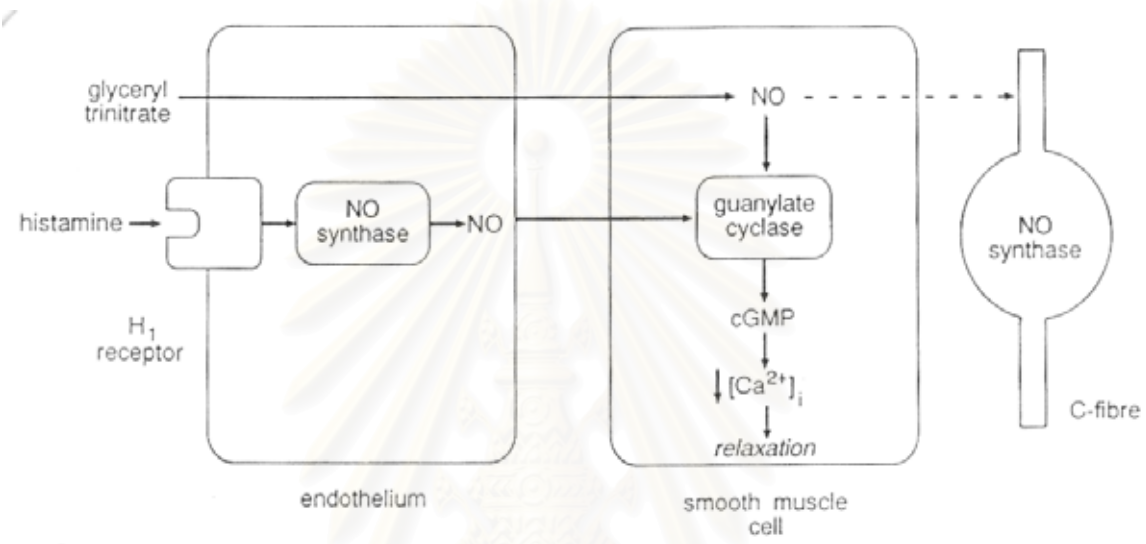


Figure 2.5 *Possible mechanisms of NO-induced headache. Glyceryl trinitrate diffuses through cerebral arterial endothelium and in smooth muscle cells it is converted to NO by mechanisms that are independent of NO synthase. Histamine, by interaction with the histamine H_1 receptor, activates NO synthase, which catalyses the formation of NO in the endothelium. NO then diffuses into vascular smooth muscle. NO from any source binds to the haeme moiety of guanylate cyclase and starts a chain of reactions that result in smooth muscle relaxation. It is unknown whether NO also reaches the perivascular nerves where, theoretically, it might excite C-fibers that project to the trigeminal ganglion. $[Ca^{2+}]i$, cytosolic Ca^{2+} concentration (Olesen, 1994).*

The fluctuations in neurotransmitter concentration both in brain and blood may trigger spontaneous migraine headache in migraine patients due to their supersensitivity to NO. Formation of NO may also be elicited by pathological reaction such as (1) spreading depression of Leao (Goadsby et al., 1992), (2) activation of the trigeminovascular system with liberation of , e.g., substance P, (3) fever and inflammation via interleukines and histamine, ect. (Olesen et al., 1994). At present, it is not known in further detail how activation of the NO pathway may cause migraine headache. Dilatation of large intra- and extracranial arteries may be involved because: (1) arterial dilatation is induced by NO liberated from endothelium and probably perivascular nerve endings (Moncada et al., 1991), (2) cephalic vasodilatation has been reported during spontaneous migraine headache (Iversen et al., 1990; Friberg et al., 1991; Thomsen et al., 1995), (3) mechanical dilatation of intracranial arteries cause referred pain in the areas where most patients feel their pain during migraine attacks, and (4) agents such as ergotamine , DHE, and sumatriptan, which constrict arteries, are effective in treatment of the acute migraine attack. On the other hand, the moderate mechanical arterial dilatation reported during migraine attacks may not be enough to cause severe pain. Another possibility is the central pain modulating effects of NO. Direct activation of perivascular sensory nerve fibers and/or initiation of privascular neurogenic inflammation (Moskowitz, 1993) by NO may be other possibilities and also the direct noxious and cytotoxic effect of NO should be considered. Whatever is true, it is striking that NO cause migraine with a delay of up to several hours (Thomsen et al., 1994), a time course that mimics the often slowly progressing development of migraine pain during spontaneous migraine attacks. As mentioned, NO is an unstable free radical with a very short half-life. Other mediators or mechanisms, therefore, seem to be involved in the rather slow cascade of events set up by activation of NO pathway and

eventually leading to a migraine attack.

There are several studies, the effect of NO on blood velocity and regional cerebral blood flow (rCBF) of cerebral circulation. The increased sensitivity to NTG in migraineur was not just subjective since transcranial doppler measurements showed that blood velocity in the middle cerebral artery was significantly more reduced during NTG infusion in migraineurs than in non-migraineurs (Figure 2.6) (Thomsen et al., 1993). Regional cerebral blood flow was unchanged by NTG (Dahl et al., 1989) and hence this indicated that NTG caused increased dilatation of the middle cerebral artery in migraine sufferers.

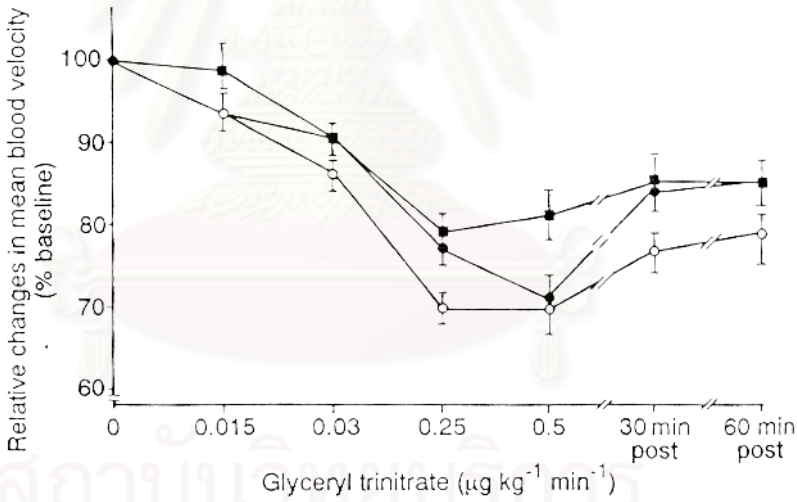


Figure 2.6 *Relative changes in mean blood velocity ± SEM in the middle cerebral artery during four ascending doses of glyceryl trinitrate infusion in human controls (closed circles), tension-type headache sufferers (close squares) and migraine sufferers (open circles) (Thomsen et al. 1993).*

However, in animal experimental, Read et al., 1997 studied the effect of glyceryl trinitrate (GTN) (0.25 ug/kg/min) for 20 min i.v. on local cerebrovascular laser Doppler flux ($rCBF_{LDF}$), artery diameter and NO concentration (selective NO microelectrode) in pial middle cerebral artery of anaesthetized cat. The results showed that GTN infusion induced a significant increase in pial artery diameter, $rCBF_{LDF}$ and NO concentration. Following termination of infusion, NO concentrations remained significantly elevated above controls for 60 min, other parameters returned to baseline within 10 min (Read et al., 1997).

SEROTONIN RECEPTORS AND THEIR FUNCTIONS

5-HT has been implicated in the aetiology of many disease states and may be particularly important in metal illness, such as depression, anxiety, schizophrenia, eating disorders, obsessive compulsive disorder (OCD), migraine and panic disorder. Indeed, many currently used treatments of these disorders are thought to act by modulating serotoninergic tone. During the last decade, multiple 5-HT receptor subtypes have been characterised. This has led to the realisation that many treatments acting via the serotonergic system, such as selective serotonin reuptake inhibitor (SSRI) antidepressants which increase presynaptic 5-HT function, or migraine prophylactics like cyproheptadine which are 5-HT receptor antagonists, have non-selective effects on postsynaptic 5-HT receptor subtypes. The development of more selective ligands may therefore lead to treatments with increased efficacy and reduced side effects. Alternatively, selective ligands may form completely novel therapies.

The serotonergic neuronal system is uniquely organized with cells of origin in the brainstem, which provide extensive projections to virtually all

areas of the brain and spinal cord. In addition, there are serotonergic neurons that originate from the midbrain raphe and innervate cerebral blood vessels. When activated these neurons change cerebral blood flow (Lance, 1992). Specific 5-HT receptor subtypes are localized to the vascular structures innervated by serotonergic neurons (Manounas et al., 1992).

In the CNS, 5-HT appears to act as a modulator, altering the level of sensory responsiveness or motor activity but not actually mediating the responses (Boadle-Biber, 1993). 5-HT has been implicated in controlling feeding behavior, thermoregulation, sexualbehavior, sleep, and pain modulation (Leonard, 1992).

SERETONIN (5-HT) SYNTHESIS AND METABOLISM

Neurons and enterochromaffin cells systhesize 5-HT from the amino acid L-tryptophan, while platelets acquire it from the blood. The first step in biosynthesis is catalyzed by the enzyme tryptophan 5-hydroxylase (EC 1.14.16.4) (the rate-limiting enzyme), which converts L-tryptophan-to 5-hydroxytryptophan (5-HTP). 5-HTP is decarboxylated to 5-HT (serotonin) by the nonspecific aromatic L-amino acid decarboxylase (EC 4.1.1.28). In neurons, 5-HT is taken up into secretory granules and stored. In man, 5-HT is mainly oxidatively deaminated by monamine oxidase (MAO) to form 5-hydroxyindoleactetaldehyde. The aldehyde is rapidly degraded by aldehyde dehydrogenase to 5-hydroxyindol acetic acid (5-HIAA), the major metabolite of 5-HT (Silberstein, 1994).

5-HT synthesis is regulated by modulating the rate of conversion of L-tryptophan to 5-HTP. The concentration of tryptophan is subsaturating

for exogenous tryptophan leads to arise in brain levels of tryptophan and an increase in 5-HT synthesis in rats. This effect is dependent on the rate of firing of the 5-HT neuron, and does not occur if firing rates are reduced. Electrical stimulation enhances 5-HT production by increasing trytophan hydroxylase activity, most likely by enzyme phosphorylation. Activation of the somatodendritic 5-HT_{1A} autoreceptors inhibits neuronal firing and 5-HT synthesis. Activation of the terminal (5-$HT_{1B/1D}$) autoreceptor inhibits the synthesis and release of 5-HT in the absence of any effect on firing rate (Boadle-Biber, 1993).

5-HT exists in several pools, and newly synthesized 5-HT is preferentially released from the storage vesicles in response to neuronal stimulation. The action of 5-HT is mainly terminated by reuptake into the nerve terminal by the 5-HT-transporter (Boadle-Biber, 1993). 5-HT interacts with its target sites through various receptors, some of which are modulated by estrogens; most migraine drugs are believed to interact with these receptors. Many receptors have been cloned and their amino acid sequence and tertiary structure established.

Initially, receptor subtypes were characterised using pharmacological tools only. On the basis of receptor biding profiles, common secondary messenger coupling and the functional activity of liganes, four main subgroups of 5-HT receptor, termed 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4, were identified. More recently, molecular biological techniques have both confirmed this classification, in that each subgroup has been found to have relatively dissimilar protein structures, and led to the identification of novel 5-HT receptors ($5HT_{1F}$,5-HT_5,5-HT_6 and 5-HT_7) enabling them to be cloned, expressed in cultured cell lines and pharmacologically and functionally characterised (Figure 2.7). Knowledge of 5-HT receptor

cDNA sequences has also allowed antibody and antisense techniques to be employed.

Current classification of Serotonin Receptors

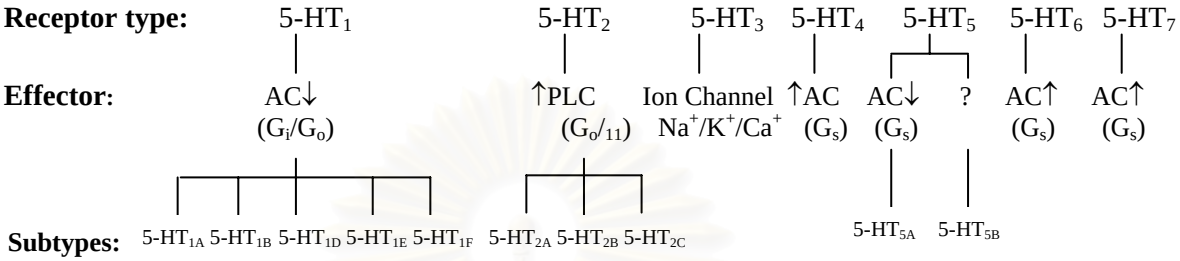


Figure 2.7 *5-HT receptors are at present divided in to 7 classes, base upon their pharmacological profiles, cDNA-deduced primary sequences and signal transduction mechanisms. With the exception of the 5-HT receptor, which forms a ligand-gated ion channel, all 5-HT receptors belong to the superfamily of G-protein coupled receptors containing a predicted seven-transmembranedomain structure (Kenett, 2000).*

THE 5-HT$_1$ RECEPTOR FAMILY

At least five 5-HT$_1$ receptor subtypes have been recognised, 5-HT$_{1A}$, 5-HT$_{1B}$ (formerly also termed 5-HT$_{1D\beta}$), 5-HT$_{1D}$ (formerly 5-HT$_{1\alpha}$), 5-HT$_{1E}$ and 5-HT$_{1F}$. All are seven transmembrane, G-protein coupled receptors (via G_i or G_o), encoded by intronless genes, of between 365 and 422 amino acids with an overall sequence homology of 40 %. These receptors are all thought to be negatively linked to adenylyl cyclase.

5-HT$_{1A}$

This receptor subtype which is located on human chromosome 5cenq 11 is widely distributed in the CNS, particularly the hippocampus septum and amygdala, areas thought to be associated with the control of mood. The receptor is negatively coupled to adenylyl cyclase, and principally causes hyperpolarization. Interestingly 5-HT$_{1A}$ receptors in the raphe nuclei, act as somatodendritic autoreceptors which inhibit neuronal cell firing and 5-HT release onto postsynaptic sites.

Activation of the postsynaptic 5-HT$_{1A}$ receptor in rats results in a characteristic 5-HT syndrome consisting of flat body posture, forepaw treading and headweaving, hypothermia and ACTH release. Stimulation of postsynaptic 5-HT$_{1A}$ receptors may also cause anxiogenic-like responses. In contrast, activation of presynaptic 5-HT$_{1A}$ receptors induces both hyperphagia and anxiolytic-like effects in rats and hence may account for the clinical anxiolytic efficacy of the 5-HT$_{1A}$ receptor agonists, buspirone and gepirone (Fletcher et al., 1993).

5-HT$_{1B}$

The 5-HT$_{1B}$ receptor is located on human chromosome 6q13 and is concentrated in the basal ganglia, striatum and frontal cortex. Recently, 5-HT$_{1B}$ receptor is found at smooth muscle cell of cerebral vessel (Longmore et al., 1997). The receptor is negatively coupled to adenylyl cyclase. The receptor was originally defined by its pharmacology and, due to species differences in the binding affinity of key ligands such as the β-adrenoceptor antsgonist, cyanopindolol (which has a higher affinity for the rat and mouse homologue), was thought to exist only in rodents. More recently, the amino

acid sequence of the receptor has been characterised and found to be 93% identical overall and 96% identical within the transmembrane domains with that of the 5-$HT_{1D\beta}$ receptor, a close homologue found in higher species, with similar distribution and function (Hoyer et al., 1993). Indeed, the differences in the pharmacology of these two homologues are now attributed to the mutation of a single amino acid in the transmembrane spanning region (Hoyer et al., 1993). Thus, it has recently been agreed to classify the receptors as species homologues of the same receptor termed 5-HT_{1B} (formerly 5-$HT_{1D\beta}$) and r5-HT_{1B} with the h and r prefix referring to the human and rat species respectively (Hartig et al., 1996).

Interesting in 5-HT_{1B} receptor agonists has been generated by the antimigraine properties of sumatriptan, a non selective 5-HT_{1D} and 5-HT_{1B} receptor agonist with low selectivity against other receptors in functional studies. This compound may act either via constriction-mediating 5-HT_{1B} receptors on cerebral arteries or by blocking neurogenic inflammation and nociceptive activity within trigeminovascular afferents. This latter action has been argued to be 5-HT_{1B} receptor-mediated as protein extravasation induced by trigeminal ganglion stimulation is blocked by sumatriptan, the selective 5-HT_{1B} receptor agonist.

5-HT_{1D}

The 5-HT_{1D} receptor (formerly termed 5-$HT_{1D\alpha}$) has 63% overall structural homology with the 5-HT_{1B} receptor (formerly 5-$HT_{1D\beta}$) and a 77% amino acid sequence homology in the seven transmembrane domains. The receptor is located on human gene 1p36.3-p34.3 and is found at nerve fiber around cerebral blood vessel and central nerve ending at TNC

(Longmore et al., 1997). It is negatively linked to adenylyl cyclase. Low levels of the 5-HT_{1D} receptor mRNA are found in the rat brain, predominantly in the caudate putamen, nucleus accumbens, hippocampus and cortex, but also in the dorsal raphe and locus coeruleus. It has been proposed that neurogenic inflammation and nociceptive activity within trigeminovascular afferents may be 5-HT_{1D} receptor.

5-HT_{1E}

The 5-HT_{1E} receptor was first characterised in man as a [^{3}H]-5-HT binding site in the presence of 5-carboxyamidotryptamine (5-CT) to block binding to the 5-HT_{1A} and 5-HT_{1D} receptors. Human brain binding studies have reported that 5-HT_{1E} receptors (representing up to 60% of 5-HT_1 receptor binding) are concentrated in the caudate putamen with lower levels in the amygdala, frontal cortex and globus pallidus. This is consistent with the observed distribution of 5-HT_{1E} mRNA (Hoyer et al., 1993). The receptor has been mapped to human chromosome 6q14-q15, is negatively linked to adenylyl cyclase and consists of a 365 amino acid protein with seven transmembrane domains (Hoyer et al., 1993). There are no reported selective or high affinity ligands for this receptor (except for 5-HT itself) and its function is currently unknown.

5-HT_{1F}

This receptor subtype is most closely related to the 5-HT_{1E} receptor with 70% sequence homology across the 7 transmembrane domains. mRNA coding for the receptor is concentrated in the dorsal raphe, hippocampus and cortex of the rat and also in the striatum, thalamus and

hypothalamus of the mouse. 5-HT_{1F} receptor mRNA has been detected in human brain and is also present in the mensentery and uterus (Hoyer et al., 1993). The receptor is negatively liked to adenylyl cyclase. The antimigraine 5-$HT_{1B/1D}$ agonist, sumatriptan, has almost equal affinity for the 5-HT_{1F} (pKi 7.6) (Adham et al., 1996) as the 5-$HT_{1B/1D}$ receptors (pK 8.4. 8.1 respectively). Thus, it has been hypothesised that the 5-HT_{1F} receptor might be a target for drugs with antimigraine properties. 5-HT_{1F} mRNA has been detected in the trigeminal ganglia whose stimulation leads to plasma extravasation in the dura a component of neurogenic inflammation which is thought to be a possible cause of migraine (Phebus et al., 1996). The first 5-HT_{1F} receptor selective agonist, LY 334370 (pk_D 9.4) with >100 fold separation over the 5-$HT_{1B/1D}$ receptors has been claimed to block the effects of trigeminal nerve stimulation as dose sumatriptan.

THE 5-HT_2 RECEPTOR FAMILY

The 5-HT_2 receptor family consists of three subtypes termed 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}. The latter site was previously termed 5-HT_{1C} before its structural similarity to the 5-HT_2 family members was recognised. All three are single protein molecules of 458-471 amino acids with a overall homology of approximately 50% rising to between 70-80% in the seven transmembrane domains. All three are thought to be linked to the phosphoinositol hydrolysis signal transduction system via the α subunit of the Gq GTP binding protein, although it is yet to be proven that the 5-HT_{2B} receptor is so coupled in native tissue (Hoyer et al., 1993). Indeed, in human pulmonary artery endothelial cells, 5-HT_{2B} receptor stimulation causes intracellular calcium release via a mechanism independent of

phosphatidylinositol hydrolysis (Ullmer et al., 1996). A similar mechanism has been detected in the rat stomach fundus (Cox and Cohen, 1996). In neither tissue was the 5-HT_{2B} receptor coupled to phosphatidylinositol hydrolysis.

5-HT_{2A}

This receptor (previously termed 5-HT_2) is located on human chromosome 13q14-q21 and is widely distributed in peripheral tissues where it mediates contractile responses of many vascular, urinary, gastrointestinal and uterine smooth muscle preparations, platelet aggregation and increased capillary permeability in both rodent and human tissue (Hoyer et al., 1993). Centrally, it is principally located in the cortex, claustrum and basal ganglia (Hoyer et al., 1993). Stimulation of central 5-HT_{2A} receptors in rodents causes head shaking and may mediate the effects of hallucinogens such as lysergic acid diethylamide (LSD) in man, the release of glutamate from the rat cerebellum and the release of β-endorphin, corticosterone, luteinising hormone and prolactin as well as adrenaline from the rat adrenal medulla (Hoyer et al., 1993).

5-HT_{2A} receptor antagonist may account for the enhanced efficacy against negative symptoms and low propensity for inducing extrapyramidal side effects of the atypical antipsychotic, clozapine, perhaps by increasing striatal dopamine release (Tricklebank, 1996). As the vasoconstrictor effects of 5-HT_{2A} receptor stimulation are markedly potentiated in hypertension and atherosclerosis, while low levels of 5-HT potentiate the thrombogenic and vasoconstrictor effects of other neurotransmitters, selective 5-HT_{2A} receptor antagonists, notably ketanserin, are in clinical use for hypertension.

5-HT$_{2B}$

This receptor located on chromosome 2q36.3-2q37.1 mediates contraction of the rat stomach fundus and endothelium-dependent relaxation of the rat and cat jugular veins and possibly of the pig pulmonary artery, via NO release (Baxter et al., 1995). 5-HT$_{2B}$ receptor mRNA has been detected throughout the mouse, rat and guinea pig colon and small intestine (Chol and Maroteaux, 1996). In man, 5-HT$_{2B}$ receptor mRNA is expressed in low levels in the brain, and at much higher levels in the placenta, lung, liver, kidney, heart, intestine and stomach. Recent receptor specific antibody studies in the rat have reported the presence of receptor protein in the amygdala, septum, hypothalamus and cerebellum (Duxon et al., 1997). In rodent studies, stimulation 5-HT$_{2B}$ receptors has been reported to cause modest anxiolysis hyperphagia and reduced grooming. They may also be involved in the precipitation of migraine and the action of the 5-HT$_2$ receptor antagonist migraine prophylactics, cyproheptadine, pizotifen and mianserin. The 5-HT$_{2B}$ receptor has also been postulated to mediate mesenteric artery contraction in hypertensive, but not normotensive rats.

5-HT$_{2C}$

5-HT$_{2C}$ specific antibodies have recently been used to show the presence of the receptor protein in the choroid plexus (highest density) and at a lower level in the cerebral cortex, hippocampus, striatum, and substantia nigra of rat and a similar distribution in man. This is consistent with previous autoradiographic studies and with mRNA distribution. There is, at present, no evidence of the existence of this receptor or its mRNA in peripheral tissues (Hoyer et al., 1993). The receptor has been mapped to

human chromosome Xq24. The presence of very high levels of the 5-HT_{2C} receptor in the choroid plexus has led to the suggestion that it may regulate cerebral spinal fluid production. It has also been hypothesised to mediate the migraine prophylactic effects of the non-selective 5-HT_2 receptor antagonist, methysergide, pizotifen, cyproheptadine and mianserin as well as the anxiogenic and panic precipitating properties of the 5-HT_{2C} agonist, mCPP in man and rats.

The 5-HT_3 receptor

The 5-HT_3 receptor binding site is widely distributed both centrally and peripherally and has been detected in a number of neuronally derived cells. The highest densities are found in the area postrema, nucleus tractus solitarius, substantia gelatinosa at all levels and nuclei of the lower brainstem such as the trigeminal nucleus and the dorsal vagal complex. It is also found in higher brain areas such as the cortex, hippocampus amygdala and medial habenula, but at lower densities (Hoyer et al., 1993). Peripherally, it is principally found on the neurons of the sensory and enteric nervous systems and pre-and postganglionic autonomic neurons. Unlike other 5-HT receptors, 5-HT_3 receptor subunits form a pentameric cation channel that is selectively permeable to Na^+, K^+ and Ca^{++} ions causing depolarization (Hoyer et al., 1993). The receptor displays marked species variation, but there is little evidence for receptor subtypes within a species, although 5-HT_3 ligands have different affinities in mouse cortex and ileum. This may be accounted for by multiple receptor binding sites (Steward et al., 1995). In vivo, administration of 5-HT_3 receptor ligands can either stimulate or inhibit cardiac function, induce vasodilation, affect

lung and intestinal function, cause pain and sensitisation of nociceptive neurons and induce nausea and vomiting.

5-HT_4 receptor

Receptor binding studies have established that the 5-HT_4 receptor is highly concentrated in areas of the rat brain associated with dopamine function such as the striatum, basal ganglia and nucleus accumbens where they may be located on GABAergic or cholinergic interneurons and/or on GABAergic projections to the substantia nigra (Palatel et al., 1995). Recently, two C terminal splice variants of 387 (short) and 407 (long) amino acids have been identified with different distributions (Gerald et al., 1995). The receptor is functionally coupled via the G-protein.

Peripherally, stimulation of 5-HT_4 receptors on the myenteric plexus of the guinea pig and rat oesophagus and guinea pig and human colon cause contractions (Bockaert et al., 1994). Thus, 5-HT_4 receptor antagonists may be of use in irritable bowel syndrome. 5-HT_4 receptors in the mucosa of the rat colon and elsewhere are involved in secretory processes (Bockaert et al., 1994). They may also mediate vomiting in ferrets, possibly by activating vagal afferents (Bockaert et al., 1994). 5-HT_4 receptors in the heart induce tachycardia and positive ontropic effects when stimulated and may be of importance in cardiovascular pathology. 5-HT_4 receptor activation may also cause cortisol secretion and bladder contraction in man (Bockaert et al., 1994).

The 5-HT_5 receptor

Two 5-HT receptors identified from rat cDNA and cloned were found to have 88% overall sequence homology, yet were not closely related to any other 5-HT receptor family (Hoyer et al., 1993). These receptors have thus been termed 5-HT_{5A} and 5-HT_{5B} and their mRNAs have been located in man. The cloned mouse 5-HT_{5A} receptor has been found to have high affinity for 5-carboxyamidotryptamine (5-CT), 5-HT, LSD and ergotamine, but little else mRNA coding for the receptor is located in rat cortex, hippocampus, habenula, olfactory bulbs and cerebellum. However, studies using a 5-HT_{5A} specific antibody have suggested that it is mainly expressed on glia cell. In cells expressing the cloned rat 5-HT_{5A} site, the receptor was negatively linked to adenylyl cyclase (Carson et al., 1995) via the G_s-protein and may act as terminal autoreceptors in the mouse frontal cortex (Pineyro et al., 1995). The rat 5-HT_{5B} coding sequence has been reported not to produce a viable protein as it is interrupted by several stop codons (Grailhe et al., 1995). Thus the 5-HT_5 receptor is poorly characterised at the present time.

The 5-HT_6 receptor

Like the 5-HT_5 receptor, the 5-HT_6 receptor has been cloned from rat cDNA based on its homology to previously cloned G-protein-coupled receptors. The rat receptor consists of 438 amino acids with seven transmembrane domains and is positively coupled to adenylyl cyclase via the G_s G-protein. The human gene has been cloned and has 89% sequence homology with its rat equivalent and is similarly coupled to adenylyl cyclase (Kohen et al., 1996). Rat and human 5-HT_6 mRNA is located in the

striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle, but has not been found in peripheral organs studied (Kohen et al., 1996).

The 5-HT_7 receptor

The 5-HT_7 receptor has been cloned from rat, mouse, guinea pig and human cDNA and is located on human chromosome 10q23.3-q24.4. Despite a high degree of interspecies homology (95%) the receptor has low homology (<40%) with other 5-HT receptor subtypes. The human receptor has a sequence of 445 amino acids and, as in other species, appears to from a receptor with seven transmembrane domains. The receptor is positively coupled to adenylyl cyclase preferentially via G_s. Rat 5-HT_7 mRNA has been observed to be most concentrated in the hypothalamus, thalamus, brainstem and hippocampus, with lower levels elsewhere. Receptor binding studies in the guinea pig are largely consistent with the mRNA distribution (To et al., 1995). In man, mRNA was also abundant in the coronary artery, descending colon stomach and ileum (Hoyer et al., 1993). Several smooth muscle relaxant responses have been characterised as 5-HT_7-like including 5-HT-induced relaxation of the precontracted guinea pig ileum, isolated rabbit femoral vein, cat saphenous vein, cynomolgous monkey and dog jugular veins while stimulation of the 5-HT_7 receptor may cause a prolonged hypotensive response in anaesthetised animal (Eglen et al., 1997). Centrally, the receptor may mediate phase advancement of neuronal activity in the suprachiasmatic nucleus and hence control circadian rythm of rats since both 5-CT and 8-OH-DPAT advance neuronal firing in hypothalamic cell cultures. In the hamster, trytophan loading increases 5-

HT release from the suprachiasmatic nucleus and modulates light-induced expression of c-fos immunoreactivity (Eglen et al., 1997).

SEROTONIN (5-HT) AND TRIGEMINAL SYSTEM

Suggestions for the involvement of serotonin in migraine date back to nearly 40 years and stem from certain seminal observations. The most convincing data demonstrated that:

- Urinary excretion of 5-hydroxyindoleacetic acid, the main metabolite of serotonin, was increased in association with migraine attacks (Curran et al., 1965; Sicuteri et al., 1961);
- Platelet 5-HT was found to drop rapidly during the onset of the migraine attack (Antrony et al., 1969);
- Intravenous injection of 5-HT aborts either reserpine-induced or spontenous headache (Kimball et al., 1960; Lance et al., 1967).

These data suggested since 5-HT drops during migraine and when infused relieves attacks, that a suitable serotonin receptor target might be identified to retain the anti-migraine effects without the unwanted effects of serotonin administration, such as, flushing, nausea, faintness, hyperpnea and paraesthesia.

Understanding the pharmacology of acute anti-migraine compounds is intimately linked to understanding the pain-producing innervation of the cranial circulation and the intracranial contents that is largely subserved by branches of the ophthalmic division of the trigeminal nerve. The effect of anti-migraine drugs upon the trigeminovascular system is the crucial link in

understanding the clinical effects of these drugs and has informed the whole range of headache problems (Table 2.1) (Goadsby, 2000).

Table 2.1.

Current concepts of the pharmacology of acute anti-migraine drugs (Goadsby, 2000).

Target	Receptor
Large cranial vessels	5-HT_{1B}
Peripheral terminal of the trigeminal of the trigeminal nerve	5-$HT_{1D/1F}$
Inhibits plasma protein extravasation	5-HT_{1D}
Blocks dural vasodilatation	5-HT_{1D}
Inhibits release of trigeminal neuropeptides	5-$HT_{1B/1D}$
Trigeminal nucleus inhibition	5-HT_{1F}

3.1 Cranial vessels and the dura mater

The role and pharmacology of the large intracranial vessels and dura mater in headache, and specifically in migraine, has been defined by the search for compounds that would mimic the effects of serotonin and ergotamine on the cranial circulation without systemic effects (Humphrey et al., 1993). The role of serotonin is discussed explicitly below. The development of sumatriptan was facilitated by the selective distribution of the ultimate target receptor in the carotid bed (Humphrey et al., 1993) and in the saphenous vein (Bax et al., 1992), which has now been confirmed anatomically using highly selective $5HT_{1B}$ and $5HT_{1D}$ receptor antibodies, to be almost exclusively of the $5HT_{1B}$ sub-type (Longmore et al., 1997; Nilsson et al., 1999). Serotonin contracts large human carotid vessels *in*

vivo (Lance et al., 1967) and has potent actions in the monkey cranial circulation (Spira et al., 1978). Sumatriptan, and its immediate predecessor AH25086B (Doenicke et al.,1987), is a potent constrictor of large cerebral vessels (Friberg et al., 1991; Perren et al., 1991) and pial vessels (Connor et al., 1992). It has, however, no effects on resting cerebral blood flow in experimental animals (Goadsby and Edvinsson, 1993; Humphrey et al., 1993) or in humans (Weiller et al.,1995).

It has been suggested that an important aspect of the effect of $5HT_{1B/1D}$ agonists is through an effect on arteriovenous shunts (Sexena, 1991). It is certainly well documented that this class of drugs closes such shunts (De Vries et al., 1997; Den Boer et al., 1992; Villalon et al., 1992), and it is primarily through this mechanism that cranial flow is redistributed after their administration. The extent to which this change contributes to the anti-migraine action is unclear, although certainly the AVA shunt model has been extremely useful in terms of dissecting the pharmacology surroundingthe *Triptans'* development.

3.2. Peripheral terminals of the trigeminal nerve

The pathway from the pain-producing structures to the level of cortical processing has a peripheral (extra-axial) component and a central dimension. The peripheral element, the trigeminal innervation of pain-producing, mainly vascular, structures (the trigeminovascular system), has been studied in considerable detail in recent years. The inhibition of trigeminal afferents in the periphery may be monitored, three ways: frist, an inhibition of neurogenic plasma protein extravasation, second, by monitoring vessels in situ, and third, inhibition of neuropeptide release.

3.2.1. Neurogenic Plasma Protein Extravasation (PPE)

Electrical stimulation of the trigeminal ganglion results in leakage of plasma proteins (Markowitz et al., 1987) from post-capillary venules (Dimitriadou et al., 1992). This plasma protein extravasation (PPE) results in a sterile inflammation in the dura mater that may explain some symptoms, particularly exacerbartion of headache by movement (Burstein et al., 1998; Strassman et al., 1994). PPE can be blocked by an array of agents including aspirin (Buzzi et al., 1989), indomethacin (Buzzi et al., 1989; Buzzi and Moscowitz, 1990), sumatriptan (Buzzi and Moskowitz, 1990), valproate (Lee et al., 1995) and neurosteroids (Limmroth et al., 1996) This effect is mediated by the $5HT_{1B}$ receptor in mice, since in genetically altered mice with no $5HT_{1B}$ receptors sumatriptan is ineffective in blocking PPE (Yu et al., 1996).

3.2.2 Intravital durally-evoked vasodilatation

Activation of dural afferents can produce two responses, plasma extravasation and dural vasomotor change. The latter has been specifically examined as an attempt to study pre-junctional trigeminal terminals. Briefly, the model involves local electrical stimulation of the dura mater through a thin bone window. Vessels, branches of the middle meningeal artery, are observed with a veideo microscropy-image measurement device to dynamically record vessel calibre. Measurements of calibre can thus be made continuously, as the dural nerves are activated, and various substances administered. It has been shown that sumatriptan and rizatriptan, both potent 5-$HT_{1B/1D}$ agonists (Goadsby, 1997) and effective acute anti-migraine compounds (Ferrari and The Subcutaneous Sumatriptan International Study Group, Ferrari, 1991; Tfelt-Hensen et al., 1998), are inhibitors of neurogenic dura vasodilatation (Williamson et al., 1997).

Moreover, in the same setting neurokinin-1, substance P, mechanisms do not play a role in vasodilatation, rather, calcitonin gene-related peptide (CGRP) is the main vasodilator transmitter (Williamson et al., 1997). By studying both trigeminal neurons and using intravital microscopy together, it has been shown that CGRP-induced vasodilatation can lead to activation of trigeminal neurons (Cumberbatch et al., 1999), offering some insight into possible mechanisms of sensitisation of dural nociceptors during migraine.

3.2.3. Inhibition of neuropeptide release

Trigeminovascular activation is marked by release of neuropeptides (Edvinsson and Goasby, 1994). The cranial circulation is innervated by three extrinsic (to the brain) systems which are: the sympathetic, parasympathetic and trigeminal systems. The sympathetic nerves are vasoconstrictor, arise from the superior cervical ganglion and are marked by neuropeptide Y (Goasby and Sercombe, 1996). The parasympathetic system is vasodilator, arises from the pterygopalatine (sphenopalatine), optic and internal carotid miniganglia, and is marked by vasoactive intestinal polypeptide (VIP) and other substances (Goadsby and Edvinsson, 1994). The trigeminovascular system is both sensory and vasodilator, arises from thetrigeminal ganglion, and is marked by SP, CGRP and neurokinin A (NKA). The distribution of the neuropeptides is summarised in Table 2.2 (Goadsby, 2000).

3.2.3.1. Calcitonin gene-related peptide.

Stimulation of the trigeminal ganglion in cats and humans results in the release of both SP and CGRP. This effect is blocked by dihydroergotamine, sumatriptan (Goasby and Edvinsson, 1994), avitriptan (Knight et al., 1997) and zolmitriptan (Goadsby and Edvinsson, 1994) but

not by CP122,288 (Gupta et al., 1995); a conformationally restricted analogue of sumatriptan (Knight et al., 1997), or 4991w93 (Giles et al., 1999), a conformationally restricted analogue of zolmitriptan (Knight et al., 1999). In this setting, CGRP release seems a good predictor of clinical outcome possibly because it refiects trigeminal activity directly. Stimulation of the superior sagittal sinus results in release of CGRP in the rat, which is attenuated by sumatriptan (Buzzi et al., 1990) and CGRP and VIP in the cat (Zagami et al., 1990). In humans during migraine, CGRP is released instead of SP (Gallai et al., 1993; Goadsby and Edvinsson, 1993; Goadsby et al., 1990); similarly, in cluster headache CGRP and VIP instead of SP release is seen (Fanciullacci et al., 1995; Goadsby and Edvinsson, 1994) and similarly in chronic paroxysmal hemicrania (Goadsby and Edvinsson, 1994).

3.2.3.2. Substance P.

In humans, thermocoagulation (Onofrio, 1975; Sweet and Wepsic, 1974) or injection of alcohol (Oka, 1950) into the trigeminal ganglion (VG) is accompanied by flushing of the skin in the distribution of the appropriate division or divisions of the nerve. Consistent with this flush, increases in skin temperature and capillary pulsation have been observed after thermocoagulation of the VG in humans for tic douloureux (Drummond et al., 1983). Cutaneous flushing and pain can be associated with local release of CGRP in patients with facial pain (Goasby et al., 1992). Stimulation of the trigeminal ganglion in humans causes release of SP, and CGRP levels are increased in the ipsilateral external jugular vein if the patient flushes (Goasby et al., 1998). However, as observed above, SP is not released in any detectable level in acute migraine (Goadsby et al., 1990). These clinical observations are consistent with the lack of effect of SP, neurokinin-1 antagonist in acute migraine (Diener, 1996; Goldstein et al.,

1997; Norman et al., 1998) and in its preventative management (Goldstein et al., 1999).

Thus, neuropeptide release is a robust method of detecting trigeminovascular activaton *in vivo*, which can be applied to humans. The finding of VIP release implicates activity of parasympathetic nerves which supports our basic observation of the presence of a functional trigeminal-autonomic loop (Goadsby and Duckworth, 1987) within the brain stem. Such a loop would explain the very marked autonomic symptomatology that accompanies certain primary headaches, such as, cluster headache and paroxysmal hemicranias (Goadsby and Lipton, 1997) and is certainly seen in some patients with migraine (Goadsby et al., 1990).

3.3. The trigeminal nucleus

Electrical (Kaube et al., 1993) or mechanical (Hoskin et al., 1996; Kaube et al., 1993) stimulation of the sagittal sinus or dural vessels (Davis and Dostrovsky, 1986) in the cat; and electrical stimulation of the sinus (Goasby and Hoskin, 1998), or middle menigeal artery in the monkey (Hoskin et al., 1999) or cat (Davis and Dostrovsky, 1988; Hoskin et al., 1999) results in the activation of a group of cells in the in the TNC and the superficial laminae of the dorsal horn of the C1 and C2 spinal cord, the *trigeminocervical complex*. It has also been shown that metabolic activity arising from stimulation of clearly ophthalmic nerve (trigeminal) innervated structure, superior sagittal sinus (Goadsby and Zagami, 1991), and the greater occipital nerve, a branch of C_2 (Goadsby et al., 1997), has a considerable degree of overlap in the trigeminocervical complex. This overlap explains the clinical fact that primary headache often ignores peripheral cuteneous innervastion patterns. These neurons then form a taget for headache pharmacology.

Table 2.2

Extrinsic innervation of the cerebral circulation (Goadsby, 2000)

	Ganglia	Effect of stimulation on CBF	Transmitters
Sympathetic	Superior cervical	-	Noradrenaline
Parasympathetic	Sphenopalatine Otic IC[b] miniganglia	+	Acetylcholine VIP[a] PHI(M)[c] PACAP[d] Helodermin Helospectin I and II Nitric oxide (NO)
Trigeminal	Trigeminal	+	Substance P CGRP[c] Neurokinin-A(NKA) Amylin Cholecystokinin-8 PACAP NO

[a] *Vasoactive intestinal polypeptide.*

[b] *Internal carotid.*

[c] *Peptide histidine isoleucine (methionine).*

[d] *Pituitary adenlyate cyclase activating polypeptide.*

[e] *Calcitonin gene-related peptide.*

3.3.1. Serotonin pharmacology

The trigeminocervical complex has receptors that bind [^{3}H]-dihydroergotamine (Goadsby and Gundlach, 1991). Specific binding of [^{3}H]-sumatriptan in cat (Mills and Martin, 1995), guinea pig (Waeber and Moskowitz, 1995) and humans (Pascual et al., 1996) and of [^{3}H]-zolmitriptan in the cat (Goasby and Knight, 1997) has been demonstrated in the trigeminocervical complex and provides a locus of action for $5HT_{1B/1D}$ agonists. These cells can be inhibited by anti-migraine drugs, such as, dihydroergotamine (Hoskin et al., 1996), eletriptan (Goadsby and Hoskin, 1996), naratriptan (Cumberbath et al., 1998; Knight and Goadsby, 1997), rizatriptan (Cumberbatch et al., 1997) and zolmitriptan (Goadsby and Hoskin, 1996). Sumatriptan does not inhibit the activity of these cells unless the blood brain barrier is disrupted (Kaube et al., 1993; Shepheard et al., 1995), an observation broadly consistent with the lack of efficacy of subcuteneous sumatriptan given during the migraine aura (Bates et al., 1994). Given the long-standing interest in serotonin in migraine (as discuss above), it is of some importance that intravenous 5HT can block trigeminal cell firing, just as those compounds synthesised to mimic its action, at doses comparable with those used in humans (Goadsby and Hoskin, 1996). For both, serotonin (Goadsby and Hoskin, 1996) and the $5HT_{1B/1D}$ agonist naratriptan (Knight and Goadsby, 1997), the inhibition of trigeminal activity is blocked by the specific $5HT_{1B/1D}$ antagonist GR127935 (Clithew et al., 1994). Data concerning the effect of $5HT_{1A}$ agonists suggests that they play no role in inhibiting trigeminocervical transmission (Cumberbatch et al., 1998).

Considering that all these physiological studies have employed intravenous administration of compounds, it has been essential to question whether the effect of $5HT_{1B/1D}$ agonists may be at some other site than the

trigeminocervical complex. Microiontophoretic application of $5HT_{1B/1D}$ agonists, sumatriptan and zolmitriptan, as well as an ergot derivative on clearly defined trigeminovascular nociceptive neurons results in reversible inhibition of firing (Storer and Goadsby, 1997). Using this method, no non-$5HT_{1B/1D}$ agonist activity of the PPE inhibitor 4491w93 could be detected in the trigeminal nucleus (Storer et al., 1999). In humans, zolmitriptan can inhibit the auditory evoked potential (Proletti-Cecchini et al., 1997), which has a degree of serotonergic influence (Wang et al., 1991). There seem little doubt that the more lipophilic brain penetrant Triptans can effect CNS structures, and the trigeminocervical complex is an ideal target for drug action currently proven for the $5HT_{1B/1D}$ receptor class agonists.

3.3.2 Other receptor systems

The trigeminal nucleus has a rich pharmacology which has begun to be explored. Glutamate is a major source of excitatory transmission within the central nervous system (Seeburg, 1993). N-Methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-metylisoxazole-4-proprionic acid (AMPA), kinate and metabotropic glutamate receptors have been identified in the superficail laminae of the TNC of the rat (Greene et al., 1993). Furthermore, the pioneering studies of Hill and Salt (Hill and Salt, 1982; Salt and Hill,1982) showed that iontophoretically applied L-glutamate excited neurons in the trigeminal nucleus caldalis. It has been shown that NMDA and AMPA antagonists can inhibit trigeminal firing (Storer and Goadsby, 1999), Fos expression (Classey et al., 1999) and local spinal cord blood flow (Goadsby and Classey, 1999) due to stimulation of the superior saggital sinus in the cat. Similarly, Fos expression in the trigeminal nucleus after administration of capsaicin can again be inhibited by NMDA (Mitsikostas et al., 1998) or AMPA blockade (Mitsikostas et al., 1999). Glutamate receptor blokade (Nicodi and Sicuteri, 1996) or NMDA

receptor modulation, such as glycine site modulation (Carignani et al., 1998; Marret et al., 1999; Miyazaki et al., 1999; Nanki et al., 1998), form an obvious target for acute intervention if adverse event can be contained.

5-HT_{2C} RECEPTOR IS A KEY FACTOR IN THE INITIATION OF MIGRAINE

During a migraine attack, platelet 5-HT decreases, urinary 5-HT increases in some patients, and 5-HIAA, a major metabolite of 5-HT, may increase. Some believe a plasma 5-HT-releasing factor may appear. These changes in 5-HT are probably epiphenomena, since changes in the plasma 5-HT levels are probably not of clinical significance in regulating cerebral arterial tone. Other evidence suggesting a role for 5-HT is based on the observation that headache can be precipitated by reserpine, which is 5-HT releasing agents, relieved by 5-HT or 5-HT_1 agonists, and blocked by treatment with methysergide (Lance, 1992; Forrari, 1989; Forrari, 1993) which is 5-HT_1 agonists.

Headaches which may be similar to migraine can be triggered by 5-HT releasing agents such as fenfluramine or reserpine and exacerbated by selective inhibition of 5-HT reuptake by drugs such as zimelodine (Fozard, 1982). A specific compound, m-chlorophenylpiperazine (m-CPP), can trigger migraine, conceivably by activation of the 5-HT_{1C} (5-HT_{2C} receptor) (Fozard, 1982).

An experimental drug tool, m-chlorophynylpiperazine (mCPP) triggers migraine in selected subjects (Brewerton et al., 1992) at dose which yield peak plasma concentrations at which the only relevant action of mCPP is agonism at 5-HT_{2C} receptors. In 1993, Gordon et al. studied 8

subjects with migraine and 10 normal control subjects each of whom was challenged either with mCPP, 0.25 mg/kg given orally, or with placebo in a double bind, crossover format. Serial measurements of serum cortisol and mCPP levels and the incidence and severity of headache were monitored. Although there were no significant differences between the migraine and normal subjects in terms of their neuroendocrine or headache responses to mCPP, there were highly significant associations between the cortisol responses and headache severity and duration (Gordon et al., 1993).The data of Gordon et al would be consistent with supersensitivity of the 5-HT_{2C} (or similar) receptor/second messenger complex being a neurochemical feature which predisposes to headache.

The measurement of 5-HT_{2C} receptors and/or their messenger RNA indicate a widespread distribution throughout the central nervous system with particularly high levels being associated with the choroid plexus (Hartig et al., 1996). In contrast, both the rat (Foguet et al., 1992) and human (Schmuck et al., 1994) 5-HT_{2B} receptor is expressed primarily in peripheral organs with only low expression levels being evident in the brain. However, with respect to the elements of the trigeminovascular system believed to be involved in migraine (Moskowitz, 1992), no pertinent information is available from radioligand or molecular biological studies. Recent data from functional studies do, however, identify a cellular location of receptors with the pharmacological characteristics of 5-HT_{2C} (or closely related) receptors and a functional response to their activations which may be relevant to the role of these sites in migraine. In rat (Bodelsson et al., 1993), pig (Sumner, 1991), and rabbit (Leff et al, 1987; Martin et al., 1993) jugular veins and pig pulmonary artery (Glusa and Richter, 1993), 5-HT, at low concentrations, induces endothelium-dependent relaxant responses; relaxation is, in the pig tissues at least,

primarily a consequence of the release of NO. Comparison of agonist relative potencies and susceptibility to blockade by antagonists indicated close pharmacological similarity to, although not identity with, the 5-HT_{2C} receptor.

Although the presence of a 5-HT_{2C} (or similar) receptor on the endothelial cells of the cerebral vessels implicated in migraine has not yet been demonstrated, it is of interest to consider the likely consequences of activation of such sites either by an exogenous agent (mCPP) or 5-HT released endogenously from neuronal terminals within the cerebral vasculature (Fozard, 1990). Firstly, and obviously, the vessel would dilate; however, as recently discussed in detail (Moskowitz, 1992a), vasodilatation is neither necessary nor sufficient to cause headache in most instances. On the other hand, the effects of endothelium derived NO (EDNO) may have consequences beyons simple vasodilation. For instance, at low concentrations, the nitrovasodilators, nitroglycerin and sodium nitroprusside, have been shown to activate sensory trigeminovascular fibers to release CGRP which in turn mediates vasodilation within the pial arteries of the cat (Wei et al., 1992). Although the precise mechanism of sensory fiber activation has not been established, it is most likely a consequence of the generation of NO from these essentially NO " donors ". Consistent with this, the release of CGRP from sensory nerves of the rabbit cutaneous vasculature induced by capsaicin has recently been shown to be NO-dependent (Hughes and Brain, 1994). It follows, therefore, that the release of EDNO by 5-HT_{2C} (or similar) receptor activation might, by sensory fiber activation and subsequent neuropeptide transmitter release, provoke the "sterile" inflammatory response on the cerebral vasculature which is believed to be a key step in the development of migraine (Fozard, 1990; Moskowitz 1992b). Consistent with this a role for endogenous NO has recently been demonstrated in acute inflammation induced by

carrageenin in the rat (Ialenti et al., 1992) and in neurogenic inflammation in guinea-pig airways (Kuo et al., 1992) and rat skin (Lippe et al., 1993). The concept of NO derived by activating 5-HT_{2C} receptors, which line on endothelial cells of the cerebral circulation, and initiate migraine are showed in Figure 2.8.

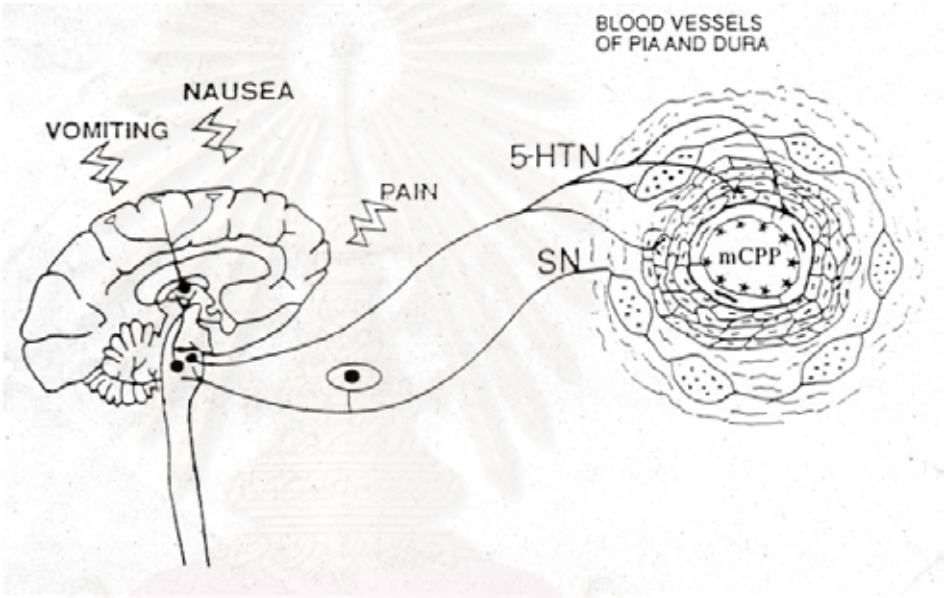


Figure 2.8 *A hypothesis for the role of 5-HT_{2C} receptors in the initiation of migraine. The central feature is the presence of 5-HT_{2C} receptors (*) on the endothelial cells lining the cerebral vasculature. Activation of these sites by mCPP or endogenous 5-HT derived from perivascular neurones arising from nuclei in the brain stem (5HTN) would induce NO release, sensory neurone (SN) activation and the initiation of the sterile inflammatory response of migraine (Forzard and Kalkman, 1994).*

CHAPTER III
SIGNIFICANCE OF PROBLEM AND OBJECTIVES

5-HT and NO are accepted to be involved in the pathogenesis of migraine. It is likely that an imbalance of the serotonergic transmitter and receptor system of the vessels is involved in the pathophysiological mechanisms of migraine. 5-HT itself causes constriction of the large ateries and dilatation of the resistance vessels. Biochemical studies have documented abnormalities of serotonergic systems in migraine. For example, plasma and platelet levels of 5-HT have been reported to vary during different phases of the migraine attack (Raskin, 1981). At the same time, increased amounts of 5-HT and its metabolite, 5-HTAA, are excreted in the urine during most headache attacks and circulating 5-HT levels were found to fall during attack. 5-HT-like substance could counteract the cerebrovascular alterations seen during a migraine attack. Intravenous injection of 5-HT agonist tends to reverse migraine headache. Hopf et al. described a patient with migraine with aura whose headaches subsided with the development of a 5-HT-secreting carciniod tumour and returned after its surgical removal (Hapf et al., 1992). Friberg et al showed that the middle cerebral artery dilatation detected during migraine attack can be reversed by sumatriptan, 5-HT_{1D} agonist. (Friberg et al., 1991). 5-$HT_{1B/1D}$ receptor agonist were used for migraine treatment. 5-$HT_{1B/1D}$ receptor were found in cerebral blood vessel, trigeminal ganglia and TNC. Recently, there are several studies show that agonist of these receptors can inhibit neuropeptide release from blood vessel and can decrease neuronal activity of TNC after chemical stimulation at dura and electrical stimulation at superior sagittal sinus, respectively (Goadsby, 2000; Nozaki et al., 1992a; Goadsby and Knight, 1997). Whereas 5-HT_{2C} receptor has recently been

suggested to play a crucial role in the initiation of migraine (Fozard and Kalkman, 1994). The vascular response to 5-HT_{2C} activation, at least in pig, is primarily a consequence of the release of NO (Glusa and Richter, 1993). 5-HT_{2C} receptor agonist (mCPP) is likely to cause vascular headache via NO synthesis (Fozard and Kalkman, 1994).

Base on several studies of headache induced by i.v. infusion of glyceryl trinitrate (exogenous nitric oxide donor) and histamine (which liberates nitric oxide from vascular endothelium), it could be suggested that NO is a key role in migraine headache. Most recently it has been confirmed in controlled double blind trials that migraineurs with a time delay of several hours (peak intensity 5.5 hours after NTG infusion) actually develop a genuine migraine attack after nitroglycerin infusion (Olesen et al., 1993; Thomsen et al., 1994). Thus, migraineurs are hypersensitive to NTG-induced headache and most likely therefore to NO. The mechanisms by which NO may trigger migraine headache are at present speculative. In theory, several neurotransmitters in brain tissue, periarterial cerebral nerves, and in the blood stimulate the formation of NO in brain neurons and arterial endothelium and possibly also interact with NOS containing nerve terminals (Moncada et al., 1991). Thus, fluctuations in neurotransmitter concentration both in brain and blood may trigger spontaneous migraine headache in migraine patients due to their supersensitivity to NO. On the other hand, the moderate mechanical arterial dilatation reported during migraine attacks may not be enough to cause severe pain. Another possibility is the central pain modulating effects of NO. Direct activation of perivascular sensory nerve fibers and/or initiation of perivascular neurogenic inflammation (Moskowitz, 1993) by NO may be other possibilities and also the direct noxious and cytotoxic effect of NO should be considered. Whatever is true, it is striking that NO

causes migraine with a delay of up to several hours (Thomsen et al., 1994), a time course that mimics the often slowly progressing development of migraine pain during spontaneous migraine attacks. As mentioned, NO is an unstable free radical with a very short half-life. Other mediators or mechanisms, therefore, seem to be involved in the rather slow cascade of events set up by activation of the NO pathway and eventually leading to a migraine attack.

Base on these finding, we performed the study for investigating the association between activation of different 5-HT receptor subtype and exogenous NO on 1. the changes of cerebral blood flow and 2. the activation of neural cell in pain processing pathway. The cerebral blood flow was studied using laser Doppler flow metry, the neural activity in pain pathway was evaluated by NOS-immunoreactivity.

Objective of this study

The aim of this study are:-

1. To study the effect of NO on nNOS immunoreactivity in trigeminovasvular system.

2. To study the effect of 5-HT receptor agonist in normal and NO-treated rats on nNOS immunoreactivity in trigeminovascular system.

3. To study the effect of NO-induced cerebral hyperemia.

4. To study the effect of 5-HT receptor agonist in normal and NO-treated rats on rCBF.

CHAPTER IV
MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Wistar rats were supplied by the National Laboratory Animal Center of Mahidol University Salaya Campus. Adult male Wistar Furth rats weigthing 250-350 grams were used in this study. The animals were housed five per cage in stainless-steel bottom cages. They were kept in a well-ventilated room in which the temperature was 28-32 °C with an automatic lighting schedule, which provided darkness from 7.00 PM to 6.00 AM. All animals were allowed access of food (Purina laboratory Chow, Premium Quality feed, Zuelig Gold Coin Mills Pte., Ltd., Singapore) and tap water ad libitum. To limit the effects of nonspecific stress, all animals were accustomed to daily handing for at least 5 days before experimentation.

CHEMICALS

- Chemical agents

Pento barbiturate sodium (Nembutal®) was purchased from Sanofi (Thailand) Ltd. Normal saline was purchased from Hospital Products Public CO, Ltd. Glyceryl trinitrate (NTG) was obtained from DBL Thailand. Sumatriptan succinate and naratriptan hydrochloride were supported from Glaxo Wellcome Research and Development, UK. 1,2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) was purchased from Research Biochemical Inc., USA. Tissue freezing medium was obtained from Jung, Germany. Bovine serum albumin (BSA), 3,3-diaminobenzidin(DAB), potassium phosphate (KH_2PO_4), tris (hydroxymethyl)-aminomethane ,tris (hydroxymethyl)-aminomethane hydrochoride and triton X-100 were purchased from Sigma, USA. Di-sodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2) and gelatin were purchased from Merck, USA. Paraformaldehyde was purchased from Electron Microscopy Sciences, USA. Sodium chloride (NaCl) and sucrose ($C_{12}H_{22}O_{11}$) were obtained from Univar, Australia. ABC streptavidin-horseradish peroxidase complex was purchased from Vector, USA.

- Antibodies

Antibodies: Rabbit antibody polyclonal agonist NOS I (NOS-I; bNOS)(Code No. AB1552; Lot No. 20020563) was purchased from Chemicon International, USA. Biotinylated swine anti rabbit IgG (Code N0. E0353; Lot No.0281301) and normal swine serum (Code No. X0901; Lot No. 110) were purchased from Dako, Denmark.

METHODS

EXPERIMENTAL DESIGN

This study plans to observe the effect of 5-HT receptor and NTG on trigeminovascular system. This study was divided 2 part. First, the laser doppler flowmetry were used to measure regional cerebral blood flow (rCBF) and second, the immunohistochemistry technique were used for determine NOS immunoreactivity.

In this study the animals were divided in to 4 groups.

1. Controlled group: The rats were injected with physiological normal saline for 5 min, intravenously.

2. NTG-treated group: The rats were injected with NTG at dose 10 mg/kg B.W. for 5 min, intravenously.

3. 5-HT receptor agonist-treated group: The rats were injected intravenously 5-$HT_{1B/1D}$ receptor agonist (sumatriptan) at dose 0.414 mg/kg B.W. and 5-HT_{1B} receptor agonist (naratriptan) at dose 0.1 mg/kg B.W. for 5 min. The effect of sumatriptan and naratriptan at these doses can decrease neuronal activity at trigeminal nucleus caudalis after chemical and electrical stimulation, respectively (Nozaki et al., 1992; Goadsby and Knight, 1997). 5-$HT_{2A/2C}$ receptor agonist (DOI) were used at dose 0.1 mg/kg B.W. for 5 min, which can express c-*fos* at secondary order neuron (Doi-Saika et al, 1997).

4. 5-HT receptor agonist with NTG-treated group: The rats were injected intravenously with 5-HT receptor agonist as same as 5-HT receptor agonist-treated group 5 min and following infused with NTG 10 mg/kg B.W. for 5 min.

To measure rCBF, 3-5 rats were used in controlled and 5-HT receptor agonist treated groups. Whereas, the datas collected from 5-8 rats in NTG and 5-HT receptor agonist with NTG treated groups. To detect NOS-IR cell, 5 rats in each group were used for immunohistochemistry technique.

REGIONAL CEREBRAL BLOOD FLOW MEASUREMENT

Surgical preparation

Experimental rats were anaesthetized for the duration of experiments by intraperitoneal administration of 60 mg/kg of sodium pentobarbital. Additional doses of anaesthetic were given as required to maintain surgical anesthesia based on testing of corneal reflex and response to tail pinch. After tracheostomy, the ventilation was assured by using positive pressure ventilator (rodent ventilator mocel 683, Harvard Apparatus, USA). A femoral artery and vein were cannulated to record blood pressure and for intravenously infusion of drugs, respectively. Blood pressure was monitored throughout experiments with pressure transducer (Nihon model TP-300T) which recorded on polygraph (Nihon RM 6000, Nihon Khoden, Japan). Arterial blood was collected periodically for determination of pH, PaO_2 and $PaCO_2$ by the pH/blood gas analyzer (278 pH/blood gas analyzer, Ciba Corning Diagnostics, UK).

Laser Doppler flow measurements.

After tracheostomy and cannulation had been performed, the rat was placed on surgical frame and the head was fixed with a stereotaxic frame. A midline incision made. The left parietal bone was then exposed by mobilization of the skin either side of the incision. The skull was exposed and

the parietal bone was thinned by drilling with saline-cooled drill, until the blood vessels of dura became clearly visible.The dura was opened by using a microneedle. A cranial window was placed over craniotomy opening. An artificial cerebrospinal fluid was infused into the intracranial space. The fiber optic needle probe of laser Doppler flowmeter (wavelength 780 nm) (Model ALF 21, Advance Co. Ltd., Japan) was fixed 1-2 mm above the cortical surface of the brain. The results of blood flow were recorded on polygraph (Nihon RM 6000, Nihon Khoden, Japan).

Data collection of rCBF

The regional cerebral blood flow (rCBF) was measured by using laser Doppler flowmetry and present in percent change from baseline. Before drug administration, control observation of blood pressure, blood gas and laser Doppler flow metry were made until a steady state was reached. After that, rCBF and MABP had been continuously recorded at each time point during drug administration and post-infusion for 20 min. Later, the result of rCBF and MABP had been read at every 5 min for 40 min.

IMMUNOHISTOCHEMICAL STUDY

Perfusion

2 hours after administration, the rats were perfused. The rats were deeply anesthetized with sodium pentobarbital. Laparotomy and thoracotomy were done. A cannula connected to a constant pressure perfusion apparatus was inserted into the apex of the heart and was advanced just distal to the aortic arch. Then, the vasculature was flushed transcardially with solution, which consisted of 300 ml of phosphate buffer saline (PBS) pH 7.4 and 2 μl heparin. The effluent should run clear and then followed by 300 ml of 4%

paraformaldehyde in 0.1 M PBS pH 7.4. The right atrium was cut to permit drainage of blood and perfusates. After perfusion trigeminal ganglia, cervical spinal cord and superior sagittal sinus were removed and post fixed in the 4% paraformaldehyde in 0.1 M PBS overnight at 4°C. Then, the tissues were changed to 30% sucrose in 0.1M phosphate buffer saline (PBS) at 4°C for cryoprotaction and were allowed to sink before sectioning.

Tissue sectioning and data collection.

For trigeminal ganglia, serial 30 μm thick sections were cut with a cryostat (Microm HM 50 N) at -20°C. All section were stained for NOS immunohistochemistry. Cell counts were carried out in 10 randomize selected section per animal. Total 100 cells were count per section then reported as a percentage of NSS-IR cell.

The cervical spinal cord was cut from obex to below obex 6 mm. in coronal plan at 30 μm of thickness and collect in a series of one in five sections. The NOS-IR neurons were counted in the area of Lamina I-II of 30 sections per animal. The average of number of NOS-IR cells per section were reported as a result.

The superior sagittal sinus was removed as whole mount preparation. The area of NOS-IR on varicosity of nerve fiber was measured by using Leica Q win program (Leica Q 500 IW, Leica imaging system Ltd., Cambridge England)

The sections were kept into 0.1 M PBS pH 7.4 at 4°C until immunohistochemical staining.

Immunohistochemical Method

Free-floating sections were rinsed in PBS 2 x 5 min, and then placed in to 1% hydrogen peroxide in PBS for 10 min to reduce the endogenous peroxidase at room temperature. The non-specific binding of the antibody was blocked by incubating tissue sections with 5% normal swine serum in PBS-A (PBS + 1% BSA + 0.3% Triton X-100) for 30 min at room temperature. Then, the section was incubated in the specific rabbit anti NOS antiserum diluted 1:500 in PBS-A at 4°C for 16-24 hours. After incubation overnight (16-24 hours), the sections were rinsed 3 x 10 min with PBS-B (PBS + 0.25% BSA +0.1% Triton X-100) and were then incubated with biotinylated swine anti rabbit IgG diluted 1:400 in PBS-B for 60 min at room temperature. After incubation, tissue sections were rinsed 2 x 10 min with PBS-B and then 1 x 10 min in PBS. The tissue sections were reacted with ABC-streptavidin horseradish peroxidase complex diluted 1:200 in PBS for 60 min at room temperature. After 1 hour, the sections were then again rinsed 2 x 10 min in PBS and 10 min in 0.05 M tris-HCl buffer (pH=7.6). Finally, they were reacted for peroxidatic activity in a solution containning 0.025% 3,3'-diaminobenzidine (DAB) and 0.01% H_2O_2 in 0.05 M tris-HCL buffer (pH 7.6) for 30 min. Then, tissue sections were washed 2 x 5 min with distilled water, mounted onto gelatinized glass slides, and coverslipped the slides with permount.

DATA ANALYSIS

All data were expressed as mean ± standard deviation (SD).The results of rCBF were presented in percent change of baseline and compare serial change by using ANOVA for repeated measurement with post hoc Dunnett's t-test. Probability values of less than 0.05 were considered to be statistically significant.

The percent of NOS-IR at trigeminal ganglia, the number of NOS-IR at lamina I-II and the area of NOS-IR on varicose were present in mean ± SD and analysis of variance (ANOVA) post hoc Bonferroni test and student t-test were used for analysis. Probability values of less than 0.01 were considered to be statistically significant.

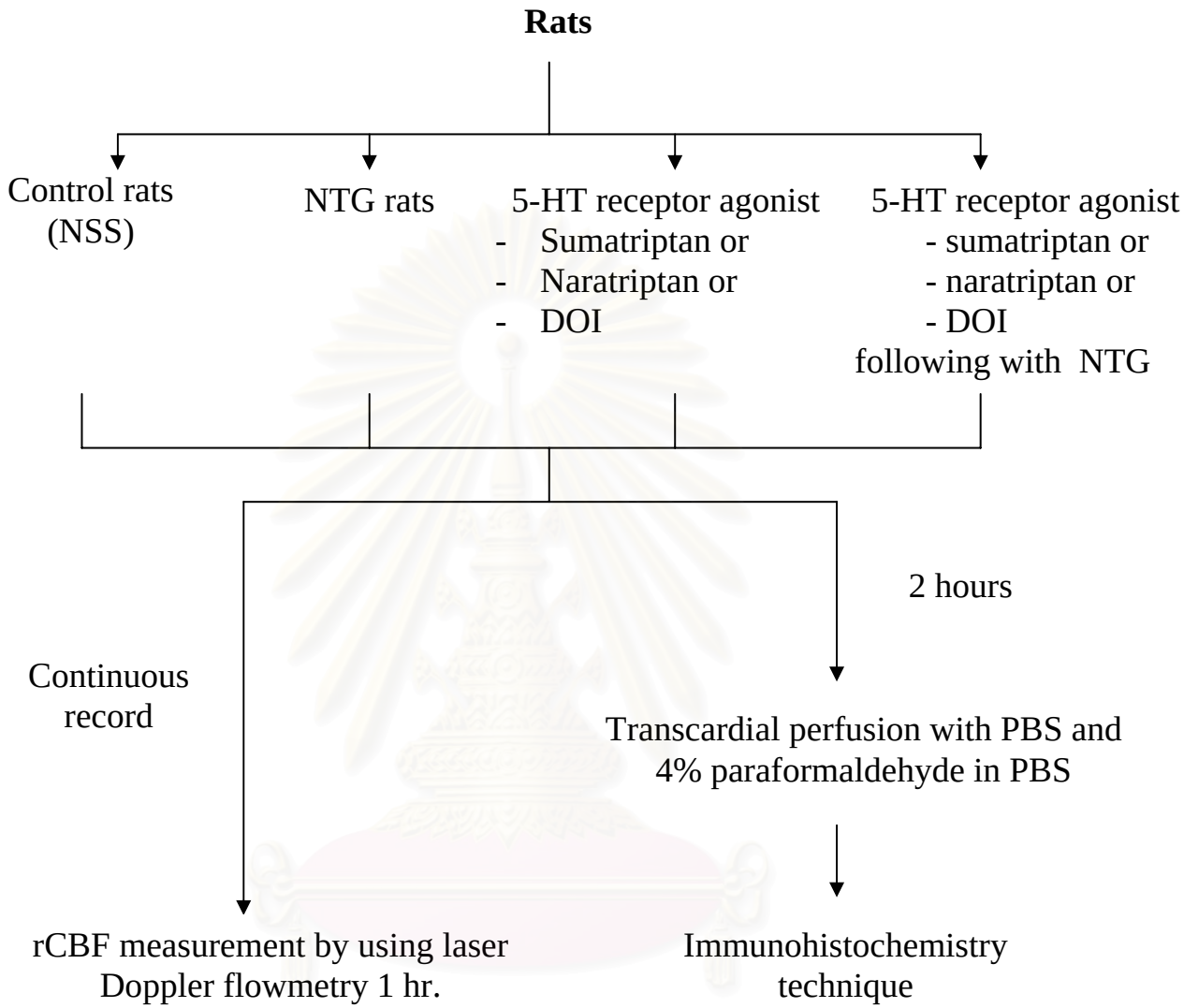


Figure 4.1. *Diagram of experimental animal groups*

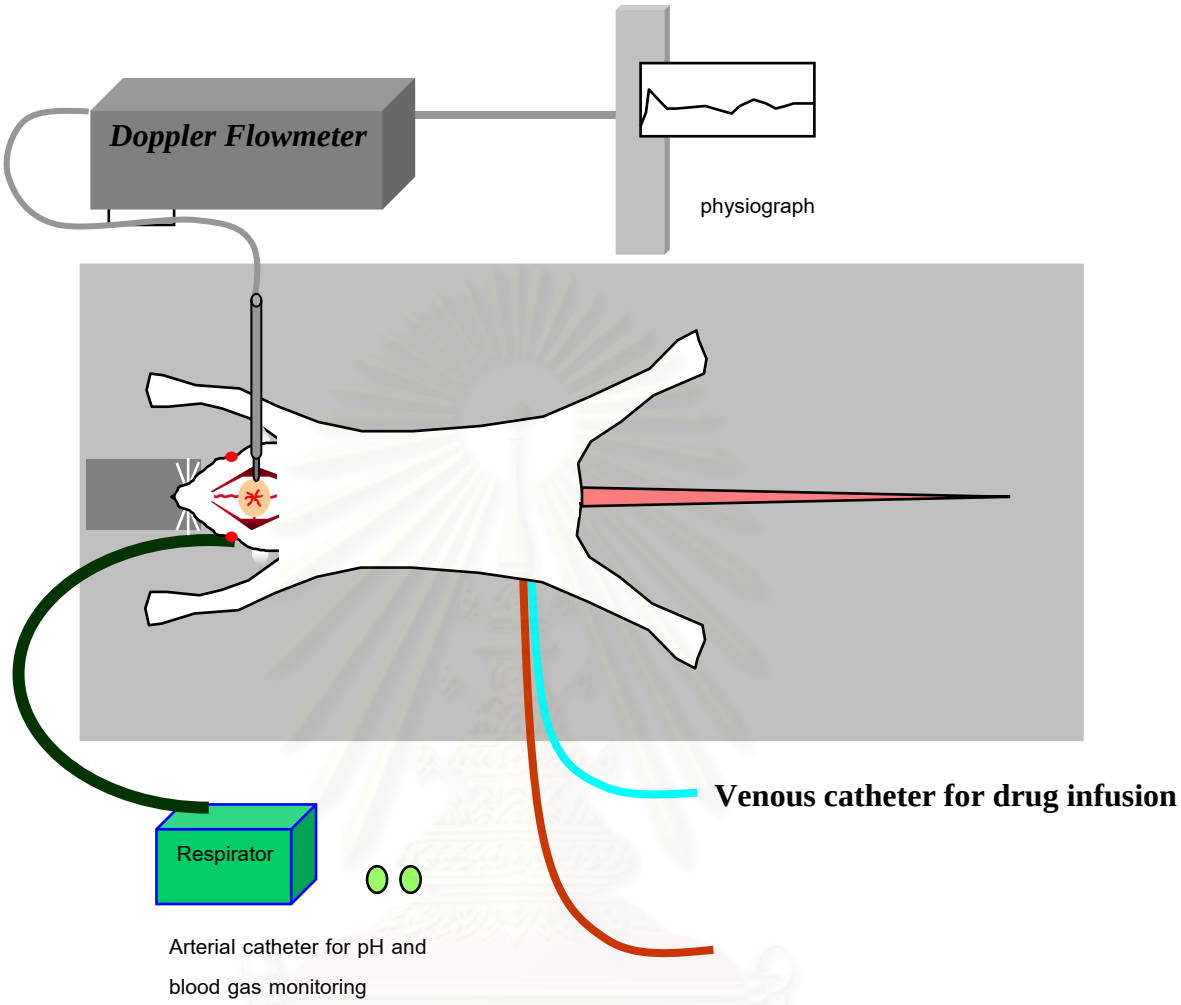


Figure 4.2 *laser Doppler flowmetry and instruments used for quantitative studies of rigional cerebral blood flow*

CHAPTER V

RESULTS

The results of this study were organized into two major parts. The first part described the effect of NTG and various 5-HT agonists on systemic blood pressure and regional cerebral blood flow. The second portion described the effect of NTG and 5-HT agonists on the expression of nNOS in neurons in the trigeminovascular pathway.

I: Effect of NTG and 5-HT agonists on the regional cerebral blood flow (rCBF)

The laser Doppler flowmetry was used to measure the rCBF. After operation, the laser probe was installed. The preparation was left until the flow recorded by flowmeter became stable. The flow averaged from five-minute recording was determined as baseline flow and was used for further calculation for each rat. All changes in the regional flow were calculated as percent change from this baseline value. After drug administration, the rCBF was recorded continuously for 60 minutes. The arterial blood pressure was also monitored during the study. The mean arterial blood pressure (MABP) was calculated from actual tracing.

The results of this part were divided into five sections as followings:

1. Effect of NTG on MABP and rCBF
2. Effect of 5-$HT_{1B/1D}$ or HT_{1B} receptor agonists on MABP and rCBF
3. Effect of 5-$HT_{2A/2C}$ receptor agonist on MABP and rCBF
4. Effect of 5-$HT_{1B/1D}$ or HT_{1B} receptor agonists on NTG-induced cerebral hyperemia.
5. Effect of 5-$HT_{2A/2C}$ receptor agonist on NTG-induced cerebral hyperemia.

The effect of NTG on MABP and rCBF

Effect on MABP.

The MABP remained stable throughout the experiment in the control (NSS-infused) group. The calculated MABP at minute 1 and 70 were found to be 84.3±11.2 and 81.7±2.9 mmHg, respectively (P=1.000). This indicated that surgical operation and monitoring did not produce any significant changes in systemic circulation.

On the other hand, substantial changes were observed in the NTG-infused group. Infusion of NTG caused instant drop in MABP. At one minute after infusion, MABP dropped from 84.3±11.2 to 48.9±19.3 mmHg. However, MABP became normalized after NTG discontinuation. The MABP at minute 11 (one minute after NTG withdrawal) was 81.8±9.5 mmHg. After this initial drop, the MABP was rather stable with minimal decline at the end of experiment. No statistically significant difference was demonstrated when MABP between the two groups were compared (ANOVA for repeated measurement)(Figure 5.1).

Effect on rCBF.

During the period of NTG infusion, the rCBF was decreased to the magnitude of ten percent. The rCBF at the end of NTG infusion was 91.0±14.4% of baseline. After this initial hyporemic phase, the rCBF became increased. The rCBF at one minute after NTG withdrawal was 139.9±38.1% of baseline value. This hyperemia was slowed decrease to the magnitude of twenty percent of baseline at minute 20 post-infusion and remained stable until the end of experiment. The observed rCBF at the end of monitoring was 116.9±18.5% of baseline. No significant change in rCBF was demonstrated in the control group. Statistical analysis showed significant difference between the two groups ($p<0.05$, ANOVA for repeated measurement)(figure 5.2).

The effect of NTG on MABP and rCBF

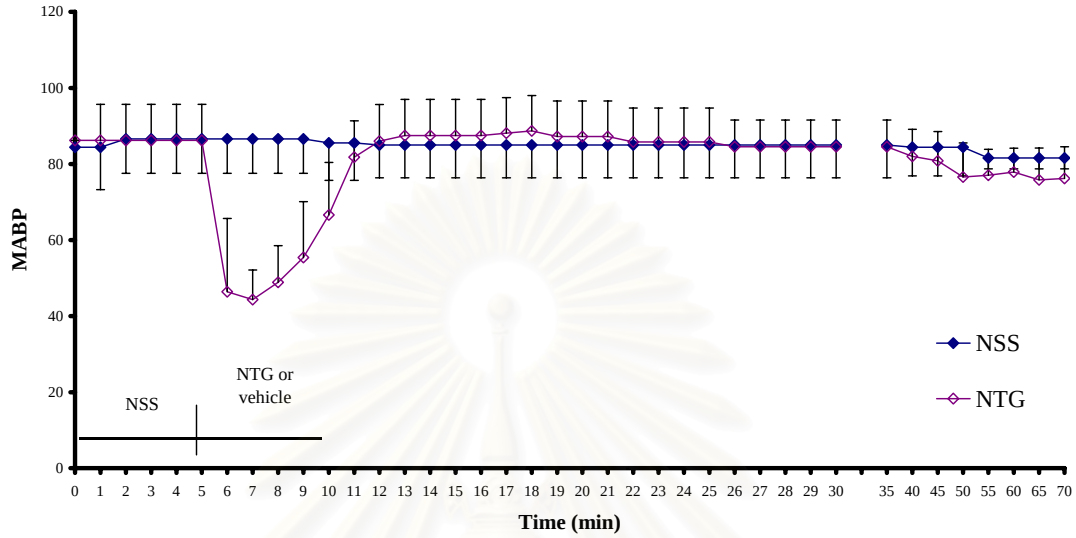


Figure 5.1 *MABP of NSS and NTG-treated group.*

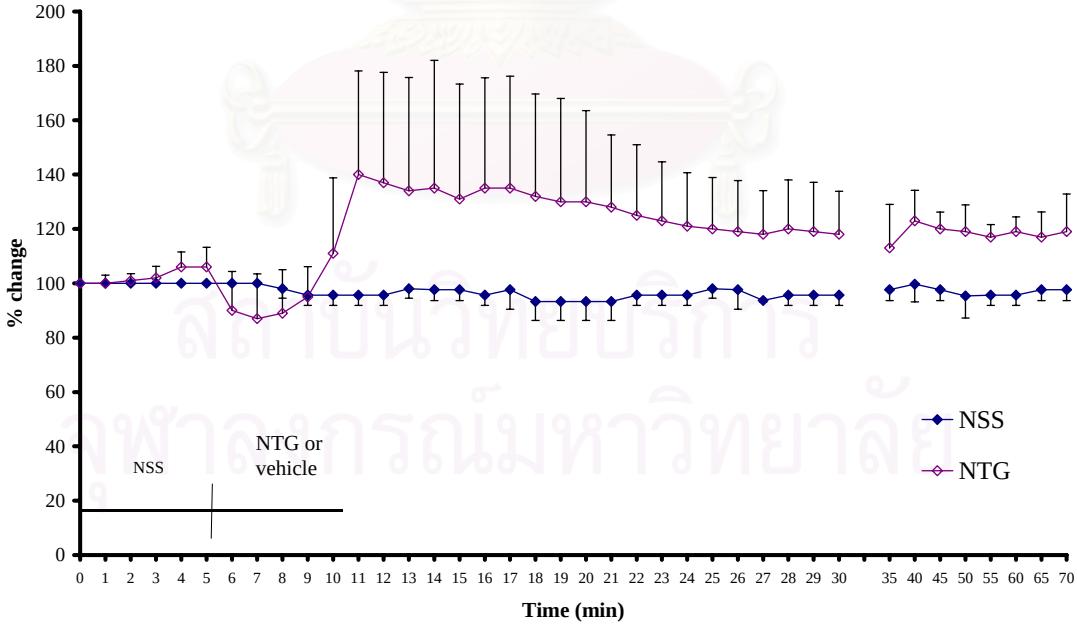


Figure 5.2 *The percent change from baseline in pial artery rCBF of NSS and NTG-treated group. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treat NSS-treated group as control ($p<0.05$).*

The effect of 5-$HT_{1B/1D}$ receptor agonist (sumatriptan) and 5-HT_{1B} receptor agonist (naratriptan) on MABP and rCBF.

Effect on MABP.

A slight and transient decrease in MABP was observed during sumatriptan infusion. The MABP decreased from 84.8±10.6 to 70.3±10.7 mmHg. After infusion, the MABP returned to 85.8±6.5 mmHg and remained stable until the end of experiment. No significant change in MABP was demonstrated in the naratriptan-treated group. Statistical evaluation revealed no significant difference among the three groups (Figure 5.3)

Effect on rCBF.

Administration of sumatriptan transiently decreased the rCBF. The rCBF at one minute post-infusion was 75.8% of the baseline value. The change became normalized when the drug was discontinued and remained stable throughout the study. The mean value of rCBF at sixty minutes after drug infusion was 97.0±12.8% of baseline. Infusion of naratriptan did not produce any significant change in rCBF (Figure 5.4)

The effect of 5-HT$_{1B/1D}$ receptor agonist (sumatriptan) and 5-HT$_{1B}$ receptor agonist (naratriptan) on MABP and rCBF.

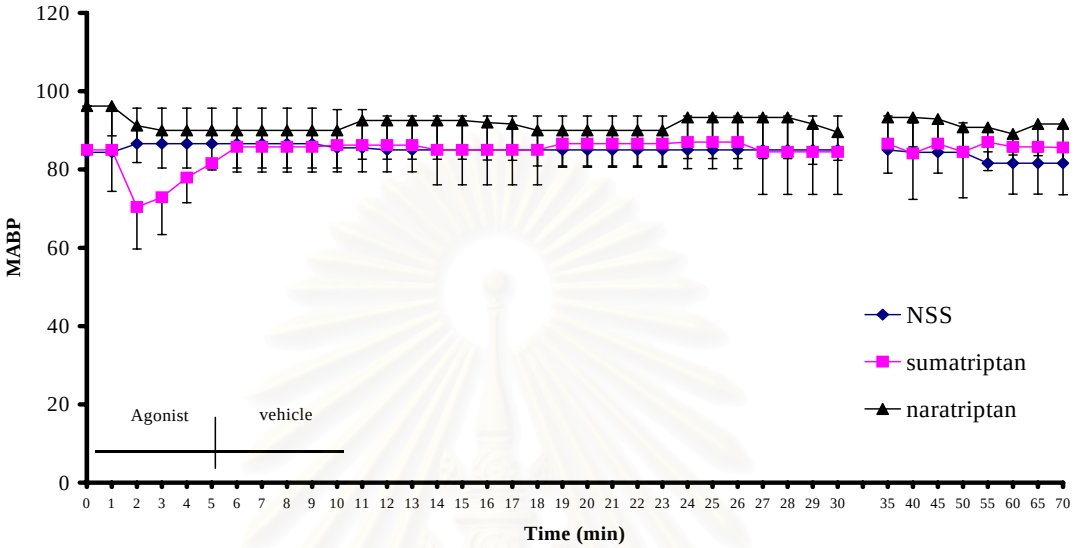


Figure 5.3 *MABP of NSS and 5-HT$_{1B/1D}$ and 5-HT$_{1B}$ receptor agonist-treated groups.*

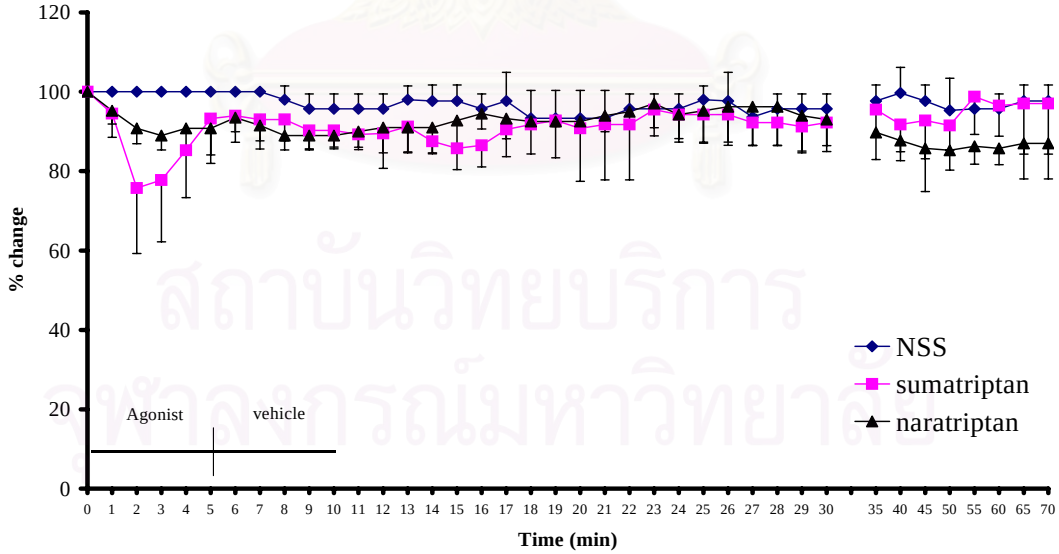


Figure 5.4 *The percent change from baseline in pial artery rCBF of NSS and 5-HT$_{1B/1D}$ and 5-HT$_{1B}$ receptor agonist-treated groups.*

The effect of 5-$HT_{2A/2C}$ receptor agonist (DOI) on MABP and rCBF.

Effect on MABP.

Administration of DOI induced a rise in MABP from 86.4±11.6 mmHg at minute 1 to 110.2±7.5 mmHg at minute 5 post-infusion. The increase in MABP was persistently observed throughout the record. The MABP at minute 60 post-infusion was 94.6 ± 19.0 mmHg. Statistical evaluation of the MABP data revealed a significant difference between the control and DOI-treated group ($p<0.05$, ANOVA for repeated measurement)(figure 5.5).

Effect on rCBF.

An elevation of the rCBF was observed in the DOI-treated group. Such change occurred almost immediately after drug infusion and was persistently observed to the end of the study. The average MABP at minute 60 after infusion was 123.2±17.8% of the baseline value. This MABP change was statistically significant as compared with data obtained from the control group ($p<0.05$, ANOVA for repeated measurement)(Figure 5.6).

The effect of 5-HT$_{2A/2C}$ receptor agonist (DOI) on MABP and rCBF.

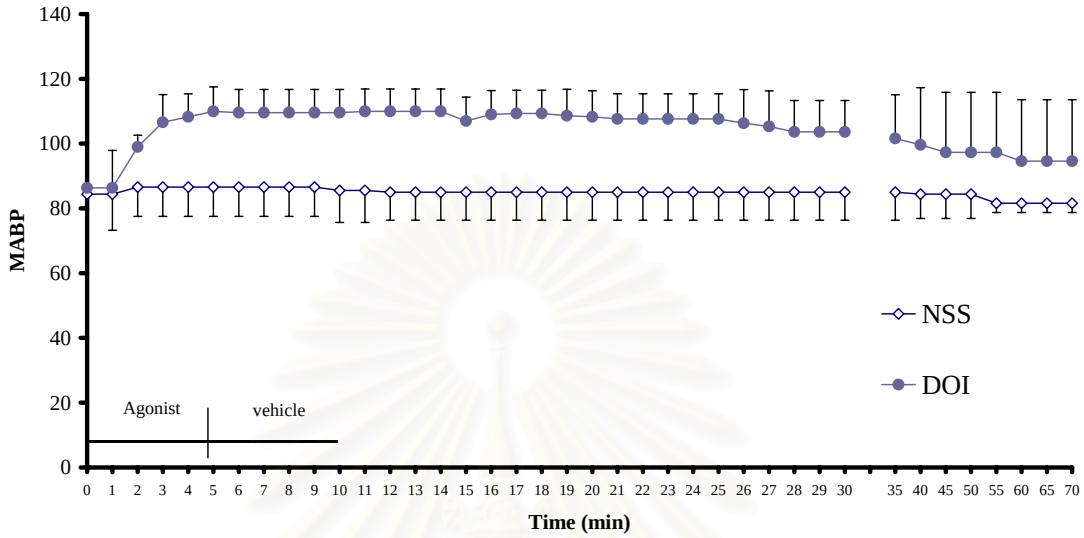


Figure 5.5 *MABP of NSS and DOI-treated group. Significant effects were assessed using ANOVA with post hoc Dunnet's t-test treat NSS-treated group as control.*

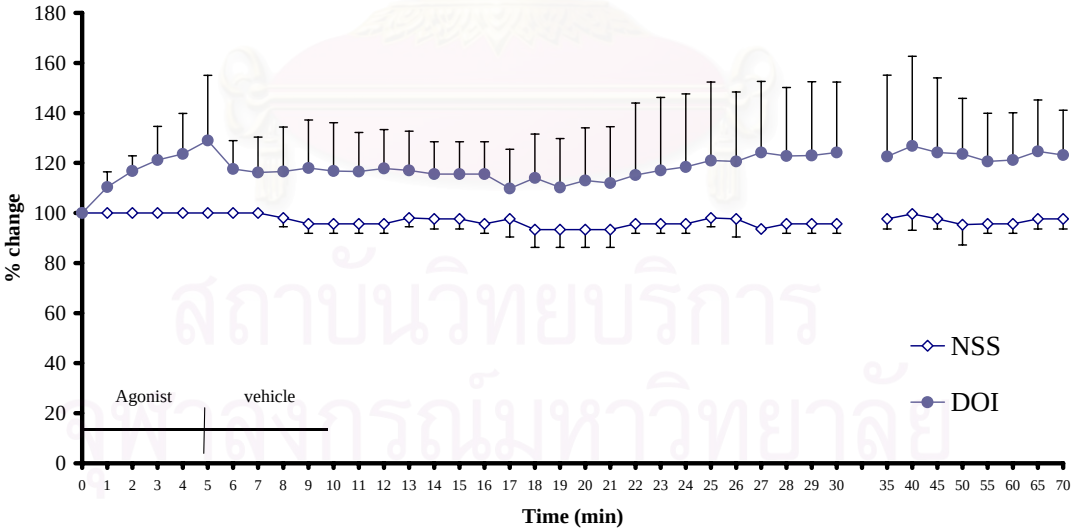


Figure 5.6 *The percent change from baseline in pial artery rCBF of NSS and DOI-treated group. Significant effects were assessed using ANOVA with post hoc Dunnett's t-test treat NSS-treated group as control.*

The effect of 5-$HT_{1B/1D}$ receptor agonist (sumatriptan) and 5-HT_{1B} receptor agonist (naratriptan) with NTG on MABP and rCBF.

Effect on NTG-induced hypotension.

Pretreatment with sumatriptan or naratriptan did not interfere with the effect of NTG in causing systemic hypotension. The magnitude of NTG-induced MABP declines in the sumatriptan-, naratriptan- and NSS-pretreated groups were 38, 34 and 35% of baseline, respectively. In the sumatriptan-pretreated group, the MABP dropped from 89.3±9.1 mmHg before NTG-infusion to 51.8±18.4 mmHg at minute 5 post-infusion. In the naratriptan-treated group, the MABP before and after NTG infusion were 82.5±12.2 and 48.4±15.9 mmHg, respectively. This initial MABP decline disappeared rapidly after NTG-discontinuation. At minute 60, the averaged MABP of sumatriptan- and naratriptan-pretreated groups were 77.7±10.7 and 79.4±12.6 mmHg, respectively. No statistically significant difference was observed when three groups were compared (Figure 5.7).

Effect on NTG-induced cerebral hyperemia.

Neither pretreatment with sumatriptan nor naratriptan could alter the effect of NTG in raising the rCBF. At 5 minutes after NTG-infusion, the rCBF of the sumatriptan- and naratriptan-treated groups were 129.7±44.4 and 135.5±23.8% of their baseline values respectively. However, it was showed that the pretreatment with sumatriptan could shorten the period of NTG-induced cerebral hyperemia. In this group, the rCBF became normalized at minute 25 post-NTG-infusion, whilst the rCBF remained increased in the NSS-pretreated group. Such normalization could not be observed in the animals pretreated with naratriptan. The rCBF at minute 60 post-infusion in the sumatriptan-, naratriptan- and NSS-treated groups were 108.3± 7.1, 127.2±21.9 and 116.9±18.5% of baseline values, respectively. However, statistical analysis did

not demonstrate any significant difference when data from the three groups were compared (Figure 5.8)

The effect of 5-HT$_{1B/1D}$ receptor agonist (sumatriptan) and 5-HT$_{1B}$ receptor agonist (naratriptan) with NTG on MABP and rCBF.

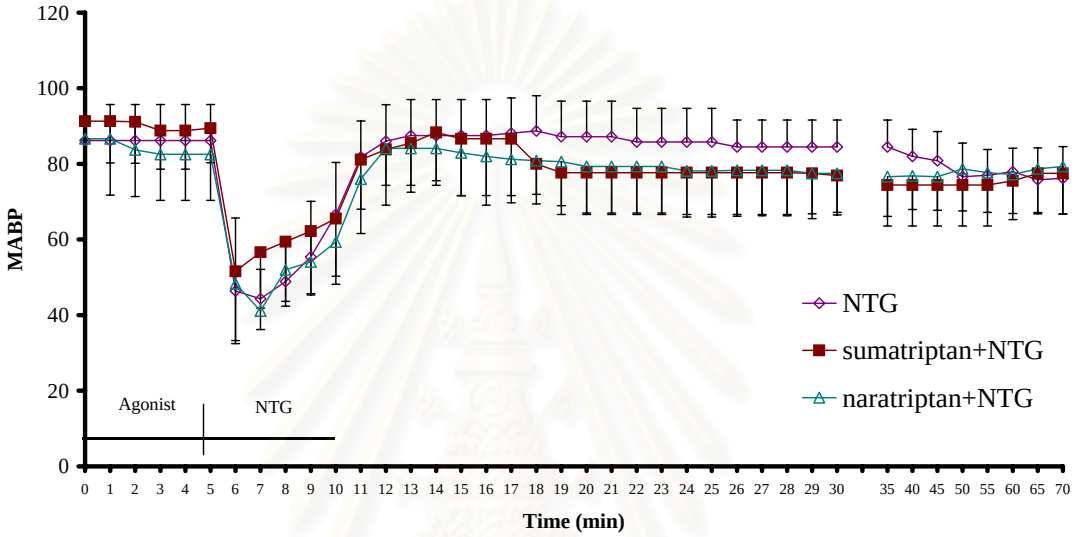


Figure 5.7 *MABP of NTG-treated group, 5-HT$_{1B/1D}$ receptor agonist (sumatriptan) and 5-HT$_{1B}$ receptor agonist (naratriptan) with NTG-treated groups.*

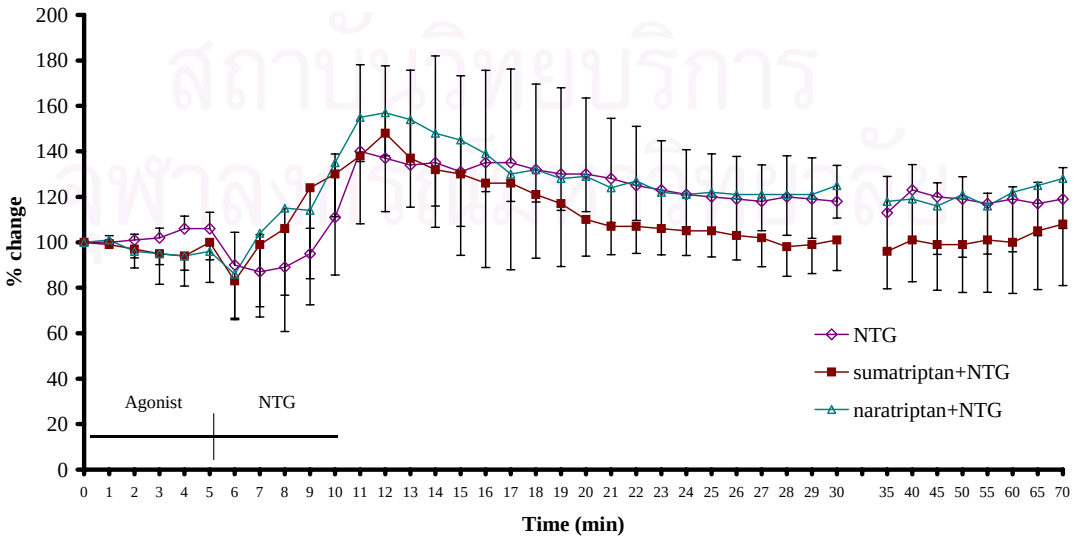


Figure 5.8 *The percent change from baseline in pial artery rCBF of NTG-treated group, 5-HT$_{1B/1D}$ receptor agonist (sumatriptan) and 5-HT$_{1B}$ receptor agonist (naratriptan) with NTG-treated groups.*

The effect of 5-$HT_{2A/2C}$ receptor (DOI) with NTG on MABP and rCBF

Effect on NTG-induced hypotension.

Infusion of DOI could minimize the effect of NTG in reducing the MABP. The MABP at minute 5 post-NTG-infusion was 74.2±13.8 mmHg, while that observed in the NSS-pretreated group was 48.9±19.3 mmHg. After NTG discontinuation, this initial MABP decline disappeared rapidly. The MABP began to rise and remained above the baseline value to the end of experiment. However, no statistical significant difference could be demonstrated (Figure 5.9).

Effect on NTG-induced cerebral hyperemia.

Pretreatment with DOI produced no effect on NTG-induced cerebral hyperemia. The rCBF was increased immediately after NTG infusion, declined at minute 25 post-infusion and remained stable at this level to the end of recording. This pattern resembled the one observed in the NSS-pretreated group (Figure 5.10).

The effect of 5-HT$_{2A/2C}$ receptor agonist (DOI) with NTG on MABP and rCBF.

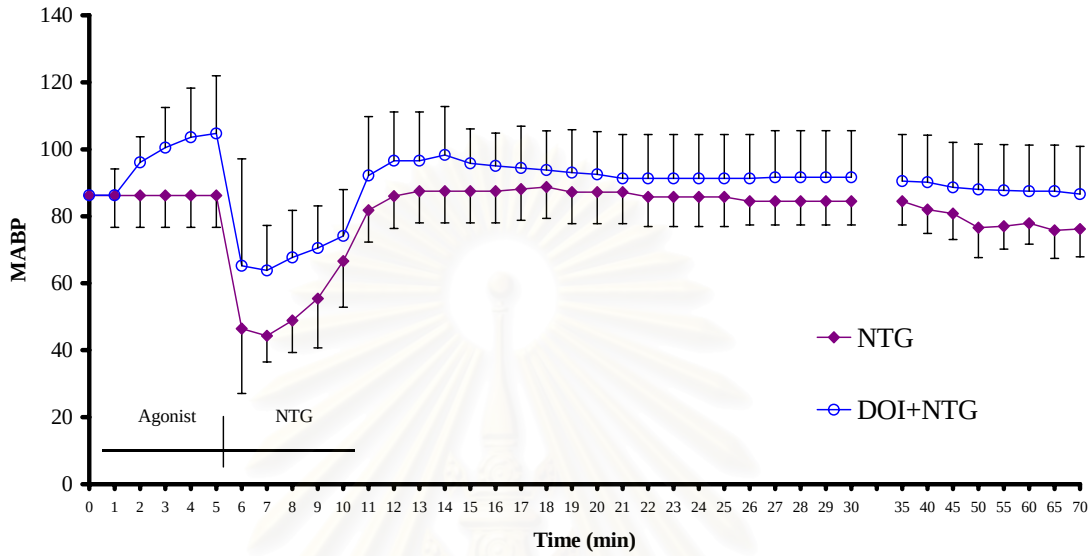


Figure 5.9 *MABP of NTG-treated group, 5-HT$_{2A/2C}$ receptor agonist with NTG-treated groups.*

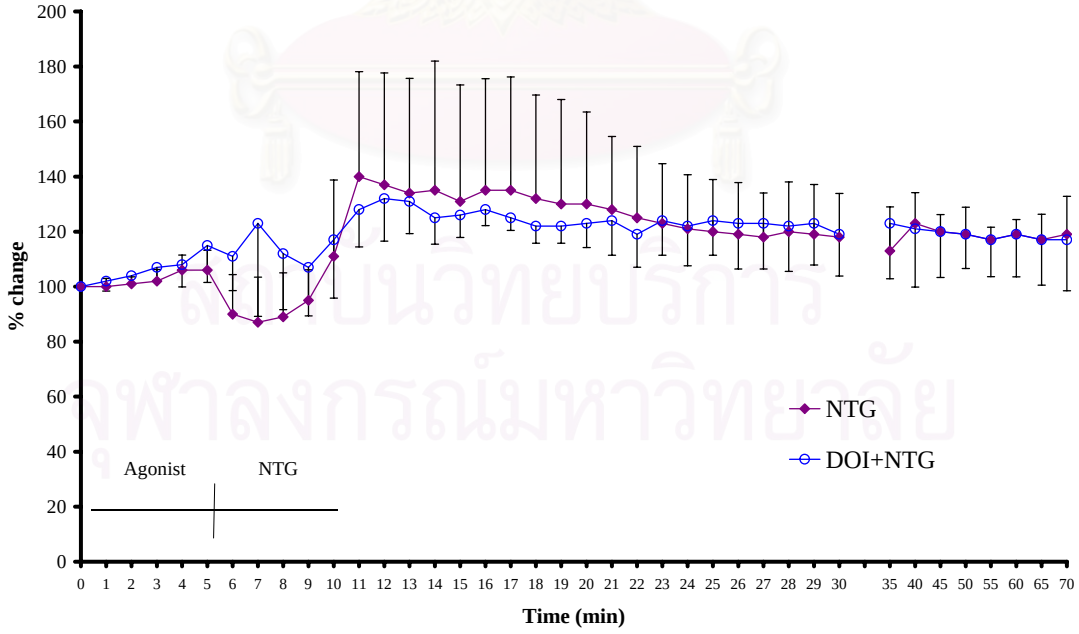


Figure 5.10 *The percent change from baseline in pial artery rCBF of NTG-treated group, 5-HT$_{2A/2C}$ receptor agonist (DOI) with NTG-treated groups.*

II: Effect of NTG and 5-HT agonists on nNOS expression in the trigeminovascular neurons

In this part, the effect of NTG and 5-HT agonists on nitric oxide system was determined using the number of nNOS immunoreactivity in trigeminovascular neurons. Three areas related to the trigeminovascular system were studied including trigeminal ganglia, TNC nucleus and perivascular nerve plexus. The expression of nNOS in the perivascular nerves was studied by calculating the surface area of nNOS-positive boutons (varicose). The nNOS-immunoreactivity in the trigeminal ganglia and TNC were described as percent of sampled neurons and cells per slide respectively.

Effects of NTG and 5-HT agonists on nNOS immunoreactivity in the trigeminal ganglia.

In this experiment, 30 sections of trigeminal ganglia were randomly chosen from each group. The slides were studied under light microscope and neurons were classified as immunoreactive or non-reactive based on the immunostaining feature. The nNOS-IR neurons were defined as those with dark-brown stained in their cytoplasm. It was showen that the nNOS-IR neurons comprised small and medium sized neurons. The large-diameter neurons were usually nNOS-negative. The total of 100 neurons was counted from each slide. Data were expressed as Mean and standard deviation of per cent of NOS-IR neurons.

Effect of NTG.

It was showed that infusion of NTG induced a significant increase in the number of nNOS-IR neurons in the trigeminal ganglia. The average numbers of nNOS-IR in NTG-treated and controls were 23.5$\pm$2.9 and 10.2$\pm$2.9 per 100 cells, respectively ($p<0.01$, student's t-test). This finding indicated that exposure to exogenous NO donor lead to the activation of endogenous nNOS enzyme in the trigeminal ganglion neurons (Table 5.1, Figure 5.11)

Effect of 5-HT$_1$ agonists.

Administration of 5-HT$_{1B/1D}$ agonist (sumatriptan) or 5-HT$_{1B}$ agonist (naratriptan) did not result in any significant change in nNOS immunoreactivity in the trigeminal ganglion neurons. The average number of nNOS-IR per 100 cells in the sumatriptan-, naratriptan- and NSS-treated groups were 10.6±2.3, 11.7± 2.5 and 10.2 ± 2.9, respectively. Statistical

evaluation revealed no significant difference among the three groups (Table 5.2, Figure 5.12).

The results showed that pretreatment with sumatriptan and naratriptan could inhibit the up-regulation of nNOS-IR evoked by administration of NTG. The number of NTG-evoked nNOS-IR in the trigeminal ganglia in sumatriptan- and naratriptan-pretreated groups were 10.4±1.9 and 14.3±3.3 per 100 cells, respectively. Statistically significant difference was demonstrated when data from both groups and those without pretreatment were compared ($p<0.01$, ANOVA) (Table 5.4, Figure 5.14).

Based on the above results, it can be concluded that although neither sumatriptan nor naratriptan can modulate the expression of nNOS in the trigeminal ganglia, both drugs can minimized the degree of NTG-evoked expression of nNOS in this structure.

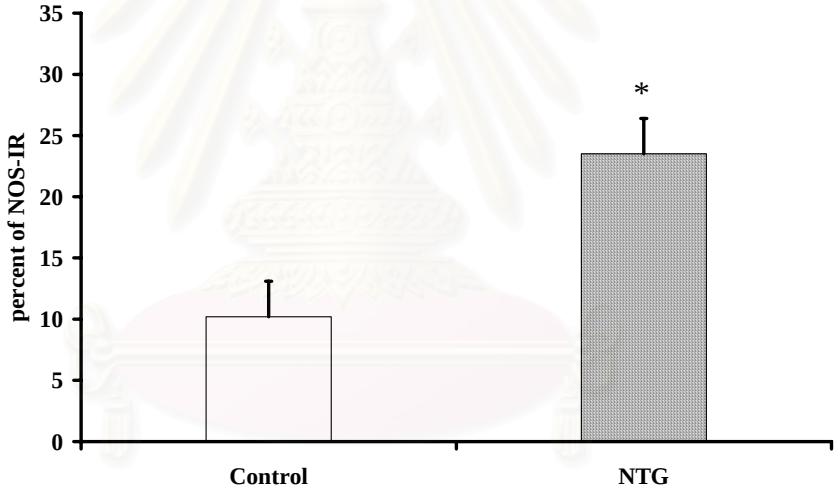
Effect of 5-HT$_2$ agonists. In contrast to those observed in the 5-HT$_1$ treated groups, the number of nNOS-IR cells in the animal treated with DOI was substantially increased. The mean value of nNOS-IR in the DOI-treated group and control were 22.0±3.5 and 10.2 ± 2.9 per 100 trigeminal ganglion neurons. The statistical analysis showed that the difference between the two groups was of significance ($p<0.01$, student's t-test) (Table 5.3, Figure 5.13).

Pretreatment with DOI did not alter the effect of NTG in inducing nNOS expression in trigeminal ganglia. The number of NTG-evoked nNOS-IR cells in trigeminal ganglia of animal with and without DOI-pretreatment were 25.2±5.4 and 23.5±2.9 neurons per 100 cells, respectively. No significant difference was evident when the two groups were compared (Table 5.5, Figure 5.15).

Table 5.1 *The mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from control and NTG-treated rats.*

Group	Mean ± SD of percent of NOS-IR
Control	10.2 ± 2.9
NTG	23.50 ± 2.9 *

* significantly different from control with p<0.01



* significantly different from control with p<0.01

Figure 5.11 *Bar graph showing the mean ± SD of the percent of NOS -IR in trigeminal ganglia obtained from control and NTG-treated rats.*

Table 5.2 *The mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from control, sumatriptan and naratriptan-treated rats.*

Group	Mean ± SD of percent of NOS-IR
Control	10.2 ± 2.9
Sumatriptan	10.6 ± 2.3
Naratriptan	11.7 ± 2.5

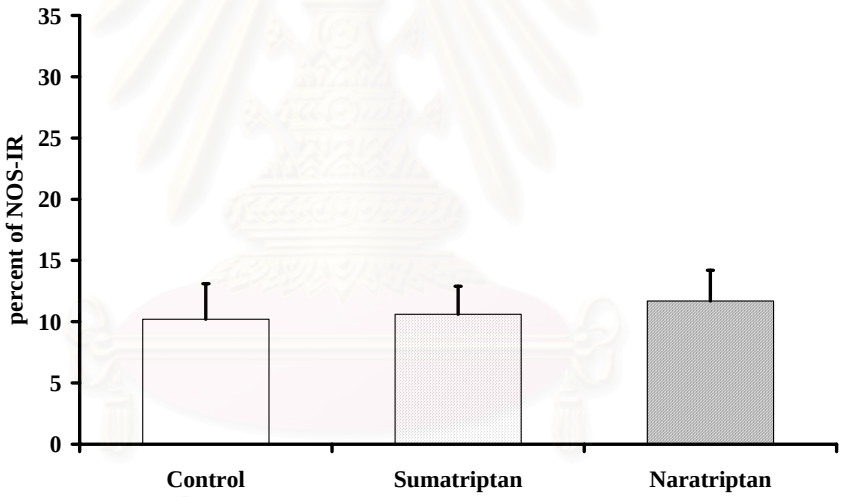
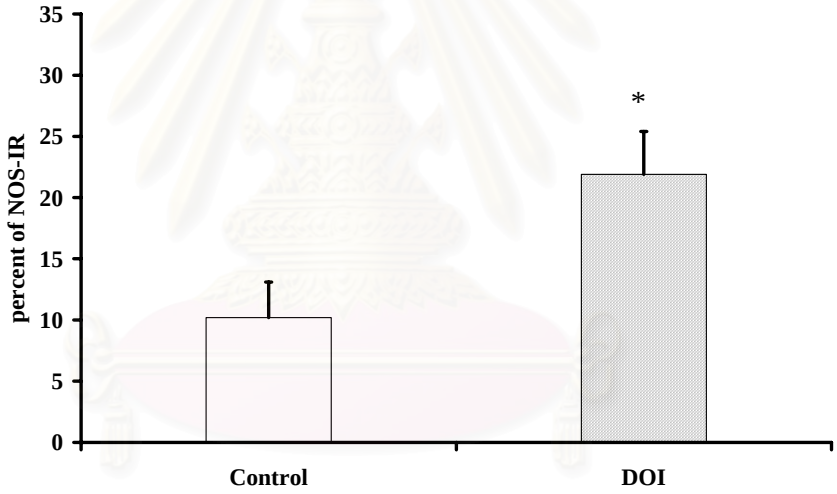


Figure 5.12 *Bar graph showing the mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from control, sumatriptan and naratriptan-treated rats.*

Table 5.3 *The mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from control and DOI-treated rat.*

Group	Mean ± SD of percent of NOS-IR
Control	10.2 ± 2.9
DOI	22.0 ± 3.5 *

* significantly different from control with p<0.01



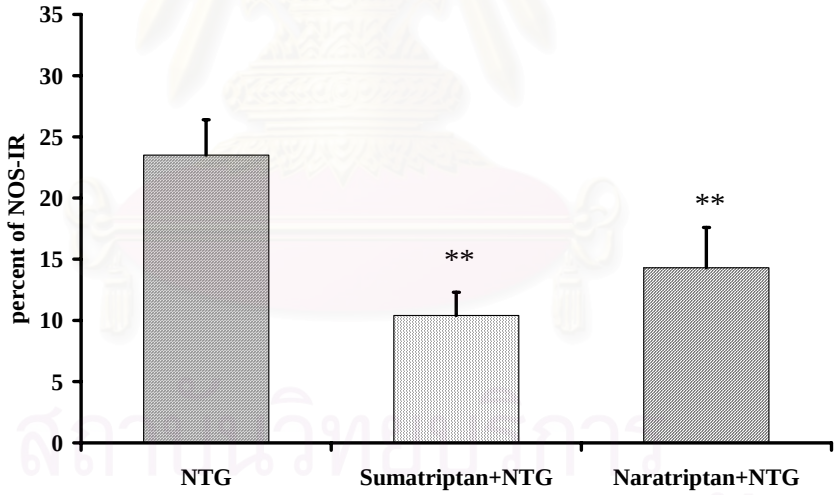
* significantly different from control with p<0.01

Figure 5.13 *Bar graph showing the mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from control and DOI-treated rats.*

Table 5.4 *The mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from NTG, sumatriptan and naratriptan with NTG-treated rats.*

Group	Mean ± SD of percent of NOS-IR
NTG	23.5 ± 2.9
Sumatriptan + NTG	10.4 ± 1.9 **
Naratriptan + NTG	14.3 ± 3.3 **

** significantly different from NTG with p<0.01



** significantly different from NTG with p<0.01

Figure 5.14 *Bar graph showing the mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from from NTG, sumatriptan and naratriptan with NTG-treated rats.*

Table 5.5 *The mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from NTG and DOI with NTG-treated rat.*

Group	Mean ± SD of percent of NOS-IR
NTG	23.5 ± 2.9
DOI + NTG	25.2 ± 5.4

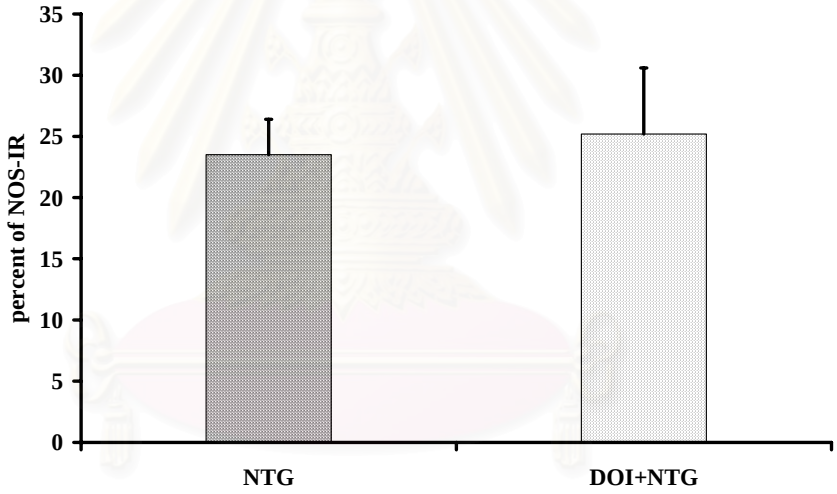


Figure 5.15 *Bar graph showing the mean ± SD of the percent of NOS-IR in trigeminal ganglia obtained from from NTG and DOI with NTG-treated rats.*

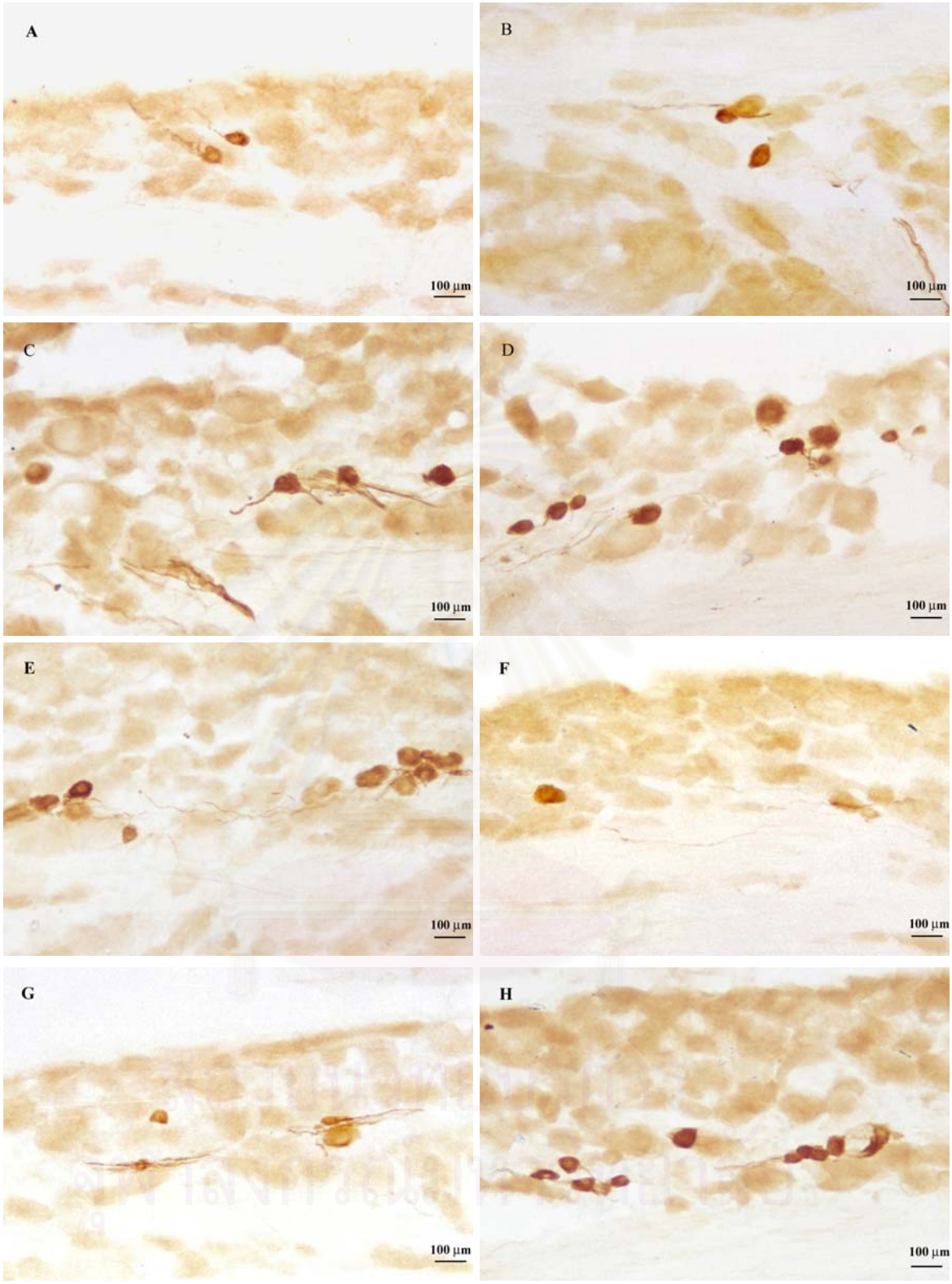


Figure 5.*16* *The photomicrograph showing the NOS immunoreactivity in trigeminal ganglia of A) NSS-treated, B) sumatriptan-treated, C) naratriptan-treated, D) DOI-treated, E) NTG-treated, F) sumatriptan with NTG treated, G) naratriptan with NTG-treated and H) DOI with NTG-treated rats.*

Effects of NTG and 5-HT agonists on nNOS immunoreactivity in the TNC.

The nNOS-IR neurons were counted from both sides of the TNC. Ten slides were randomly selected from each rat and the nNOS-IR cells were counted. Number of counted nNOS-IR cells from each group were then average was reported as mean and standard deviation.

Effect of NTG.

The administration of NTG resulted in a substantial increase in the number of nNOS-IR neurons in the TNC. The number of nNOS-IR cells raised from 29.6±14.6 per section in the control group to 52.4±27.2 cells per section in the NTG-treated group ($p<0.01$, student's t-test) (Table 5.6, Figure 5.17).

Effect of 5-HT$_1$ agonists. Neither sumatriptan nor naratriptan could alter the number of nNOS-IR in the TNC. The numbers of nNOS in the TNC in sumatriptan-, naratriptan- and NSS-pretreatment were 33.2±18.3, 36.2±17.6 and 29.6±14.6 cells per section, respectively. No statistical significance was evident when these three groups were compared (Table 5.7, Figure 5.18).

Pretreatment with sumatriptan could minimize the NTG-evoked nNOS-IR in the TNC. The number of NTG-evoked nNOS-IR cell in the sumatriptan-pretreated and control groups were 31.4±17.1 and 52.4±27.2 neurons per section, respectively. The difference between the two groups was statistically significant ($p<0.01$, student's t-test). Pretreatment with naratriptan slightly decreased the number of NTG-evoked nNOS-IR neurons in the TNC. The

number of NTG-evoked nNOS-IR in the naratriptan-pretreated animals was 47.1±27.0 neurons per 100 cells. This value did not differ significantly from the control (Table 5.9, Figure 5.20).

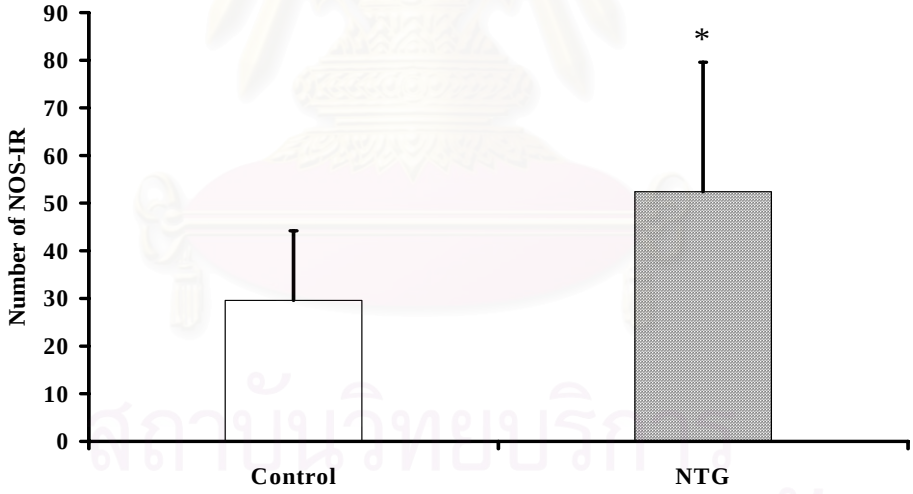
Effect of 5-HT$_2$ agonists.

Administration of DOI could enhance the expression of nNOS in the TNC. The number of nNOS-IR in the DOI-treated group was 47.6±29.0 per section, whilst the value in the control group was 29.6±14.6 per section. This value was significantly greater than the value of the control ($p<0.01$, student's t-test) (Table 5.8, Figure 5.19). However, pretreatment with DOI did not interfere with the effect of NTG in up-regulating the nNOS expression in the TNC. The number of NTG-evoked nNOS-IR in the animal with and without DOI-pretreatment were 46.2±24.8 and 52.4±27.2 per section, respectively (Table 5.10, Figure 5.21).

Table 5.6 *The mean ± SD of the NOS-IR in TNC obtained from control and NTG-treated rats.*

Group	Mean ± SD of NOS-IR
Control	29.6 ± 14.6
NTG	52.4 ± 27.2 *

* significantly different from control with p<0.01



* significantly different from control with p<0.01

Figure 5.17 *Bar graph showing the mean ± SD of NOS-IR in TNC obtained from control and NTG-treated rats.*

Table 5.7 *The mean ± SD of the NOS-IR in TNC obtained from control, sumatriptan and naratriptan-treated rats.*

Group	Mean ± SD of NOS-IR
Control	29.6 ± 14.6
Sumatriptan	33.2 ± 18.3
Naratriptan	36.2 ± 17.6

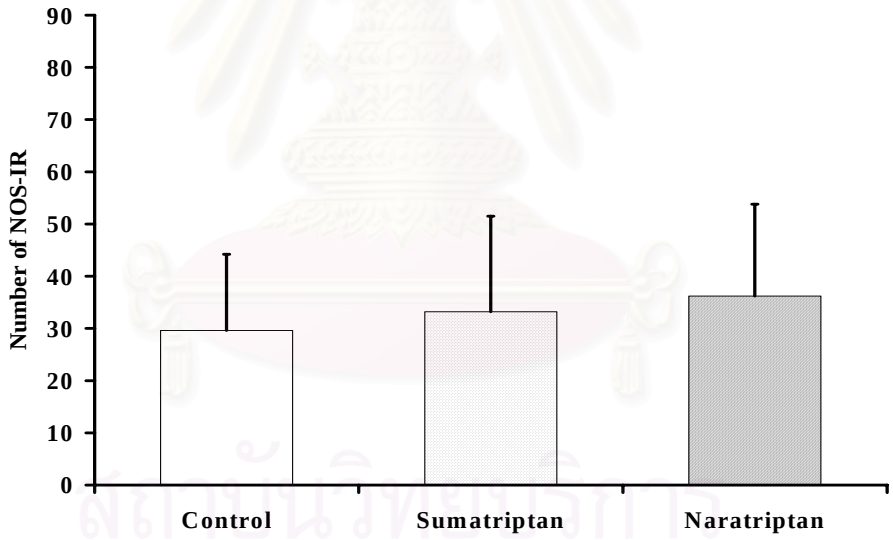
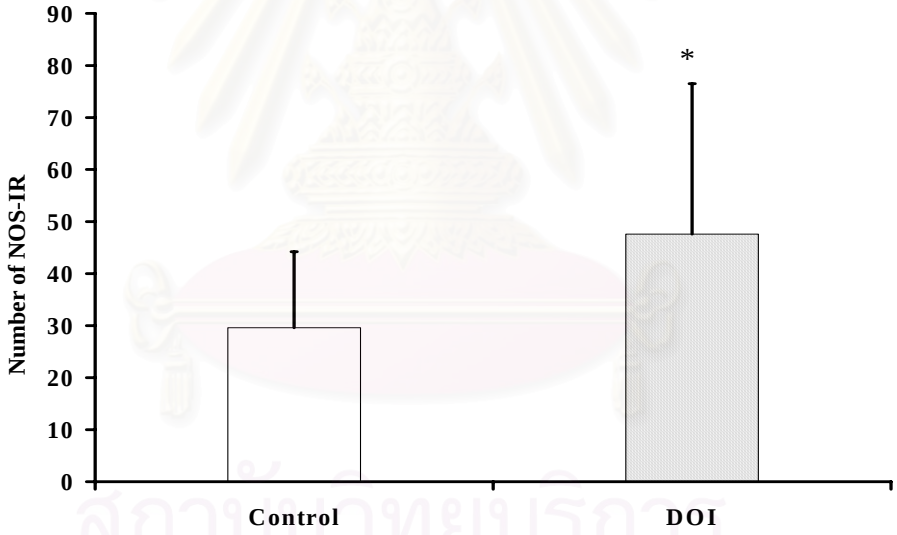


Figure 5.18 *Bar graph showing the mean ± SD of NOS-IR in TNC obtained from control, sumatriptan and naratriptan-treated rats.*

Table 5.8 *The mean ± SD of the NOS-IR in TNC obtained from control and DOI-treated rats.*

Group	Mean ± SD of NOS-IR
Control	29.6 ± 14.6
DOI	47.6 ± 29.0 *

* significantly different from control with p<0.01



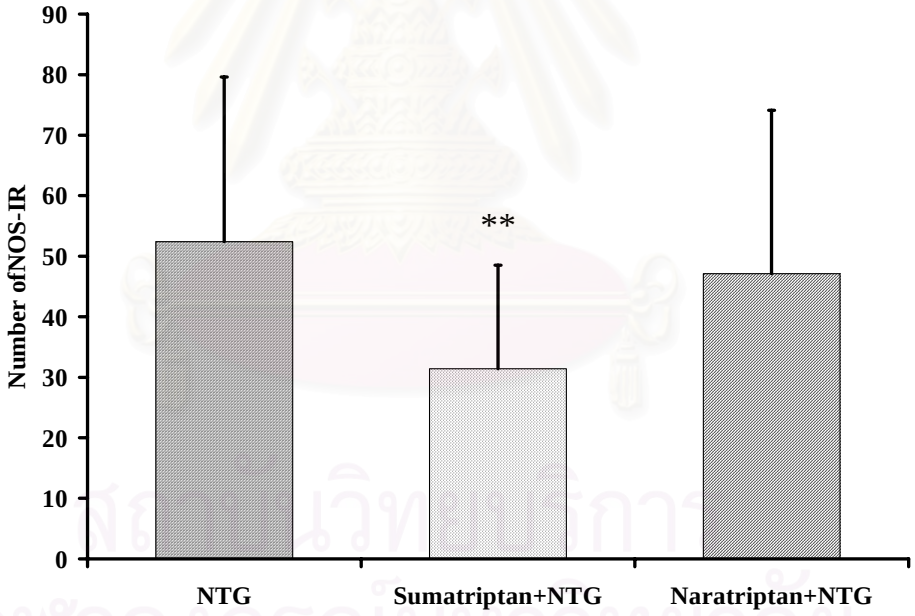
* significantly different from control with p<0.01

Figure 5.19 *Bar graph showing the mean ± SD of NOS-IR in TNC obtained from control and DOI-treated rats.*

Table 5.9 *The mean ± SD of the NOS-IR in TNC obtained from NTG, sumatriptan and naratriptan with NTG-treated rats.*

Group	Mean ± SD of NOS-IR
NTG	52.4 ± 27.2
Sumatriptan + NTG	31.4 ± 17.1 **
Naratriptan + NTG	47.1 ± 27.0

** significantly different from NTG with p<0.01



** significantly different from NTG with p<0.01

Figure 5.20 *Bar graph showing the mean ± SD of NOS-IR in TNC obtained from NTG, sumatriptan and naratriptan with NTG-treated rats.*

Table 5.10 *The mean ± SD of the NOS-IR in TNC obtained from NTG and DOI with NTG-treated rats.*

Group	Mean ± SD of NOS-IR
NTG	52.4 ± 27.2
DOI + NTG	46.2 ± 24.8

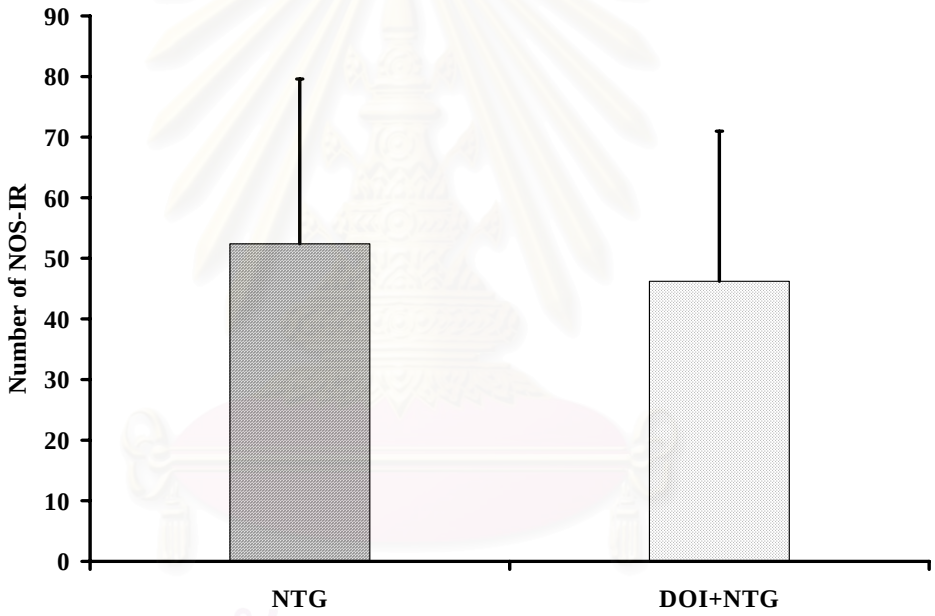


Figure 5.21 *Bar graph showing the mean ± SD of NOS-IR in TNC obtained from NTG and DOI with NTG-treated rats.*

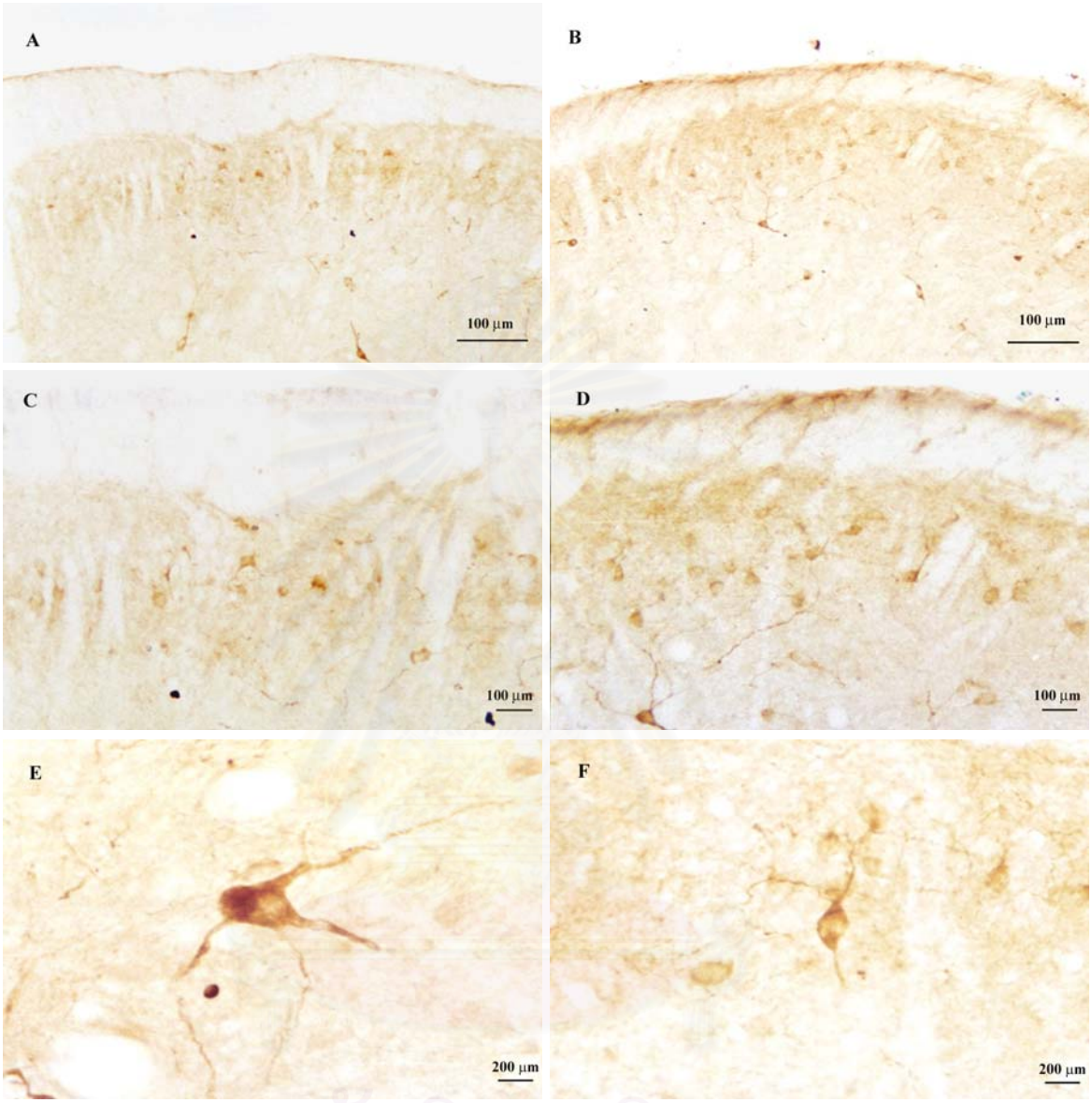


Figure 5.22 *The photomicrograph of NOS-labelling in the trigeminal nucleus caudalis of NSS-treated (A,C,E), NTG-treated rats (B,D,F). A) and B) are at low magnification whereas C) and D), and E) and F) are medium and high magnification, respectively.*

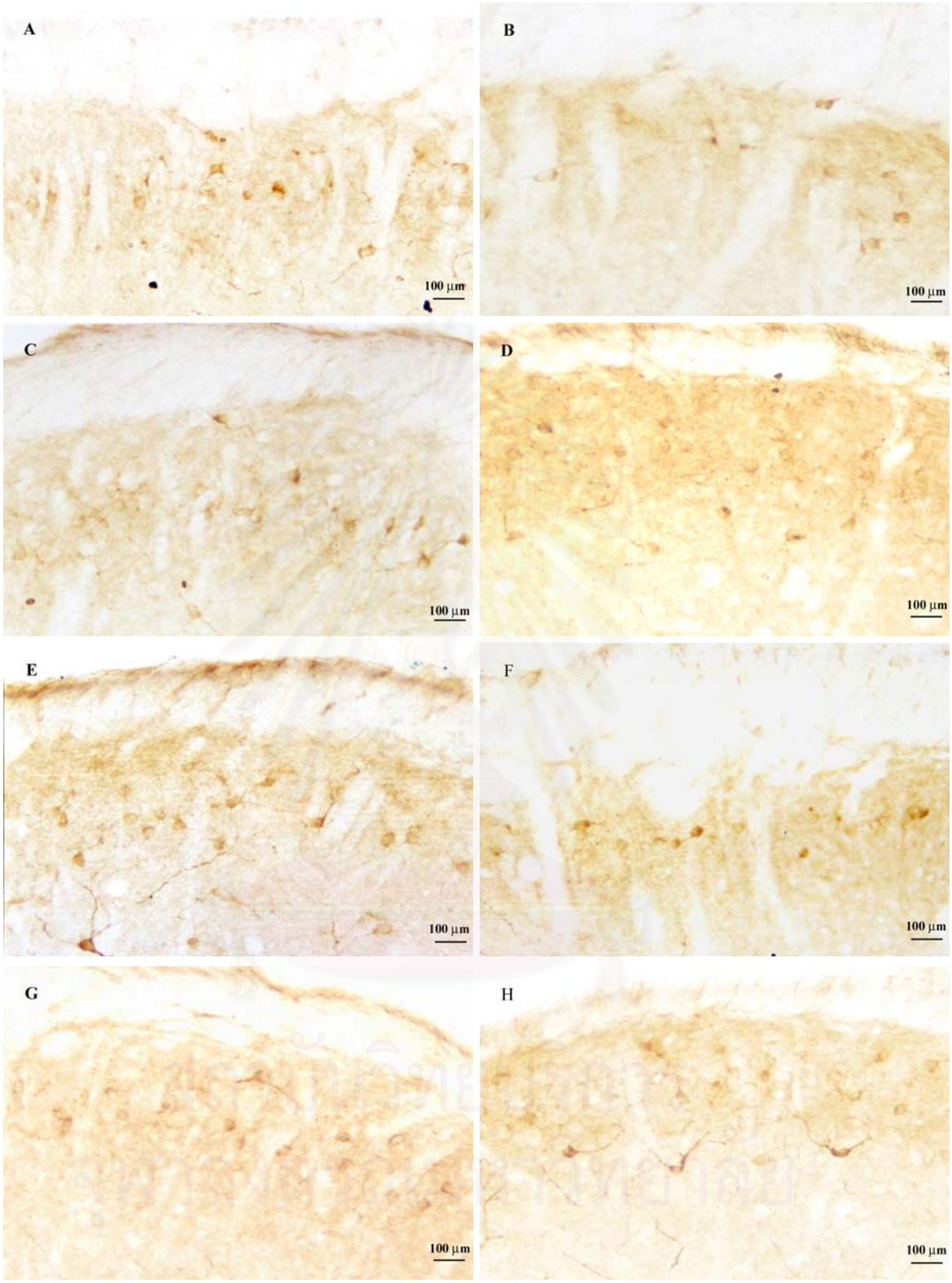


Figure 5.23 *The photomicrograph showing the NOS immunoreactivity in trigeminal nucleus caudalis of A) NSS-treated, B) sumatriptan-treated, C) naratriptan-treated, D) DOI-treated, E) NTG-treated, F) sumatriptan with NTG-treated, G) naratriptan with NTG-treated and H) DOI with NTG-treated rats.*

Effects of NTG and 5-HT agonists on nNOS immunoreactive perivascular nerve plexus of superior sagittal sinus

In this experiment, nNOS-IR nerve fibers located on the adventitial layer of superior sagittal sinus were studied. It was showed that sagittal sinus was densely innervated with nNOS-IR nerve fiber. The Size of nNOS-IR perivascular varicosity was determined and reported as square micrometer.

Effect of NTG.

It was showed that the size of nNOS-IR perivascular neural varicose was significantly greater in the NTG-treated groups as compared with the control. The averaged surface areas calculated of the varicosity were 1.9±1.2 and 1.3±1.0 μm^2 for NTG-treated and control groups, respectively ($p<0.01$, student's t-test) (Table 5.11, Figure 5.24).

Effect of 5-HT$_1$ agonists. No significant change in the size of nNOS-IR perivascular varicose was demonstrated among sumatriptan-, naratriptan- and NSS-treated groups. The size of the boutons measured from these three groups were 1.25 ± 0.8, 1.1 ± 0.6 and 1.3 ± 1.0 μm^2, respectively (Table 5.12, Figure 5.25).

Despite no effect on the nNOS-IR perivascular fiber in the resting state, it was demonstrated pretreatment with sumatriptan- or naratriptan could block the nNOS-IR varicosity enlargement evoked by NTG-infusion. The average surface of area of the boutons after NTG infusion in the sumatriptan-, naratriptan- and NSS-pretreatment were 1.45±0.9, 1.6±0.9 and 1.9±1.2 μm^2, respectively ($p<0.01$, ANOVA) (Table 5.14, Figure 5.27)

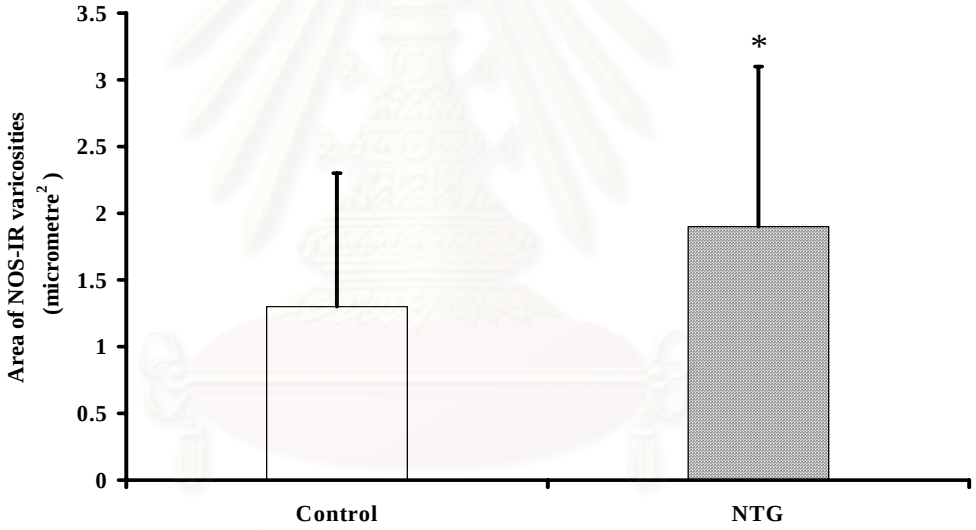
Effect of 5-HT$_2$ agonist. Administration of DOI led to a moderate (about 25% of baseline value) enlargement of nNOS-IR perivascular nerve

boutons. The difference of surface area of boutons between this group and the control was statistically significant ($p<0.01$, student's t-test) (Table 5.13, Figure 5.26). However, the magnitude of enlargement was lesser than those observed in the NTG-treated group. Pretreatment with DOI did not exert any effect on NTG-evoked enlargement of nNOS-IR perivascular boutons (Table 5.15, Figure 5.28).

Table 5.11 *The mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from control and NTG-treated rats.*

Group	Mean ± SD of area of NOS-IR varicosities (μm^2)
Control	1.3 ± 1.0
NTG	1.9 ± 1.2 *

* significantly different from control with p<0.01



* significantly different from control with p<0.01

Figure 5.24 *Bar graph showing the mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from control and NTG-treated rats.*

Table 5.12 *The mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from control,sumatriptan and naratriptan-treated rats.*

Group	Mean ± SD of area of NOS-IR varicosities (μm^2)
Control	1.3 ± 1.0
Sumatriptan	1.25 ± 0.8
Naratriptan	1.1 ± 0.6

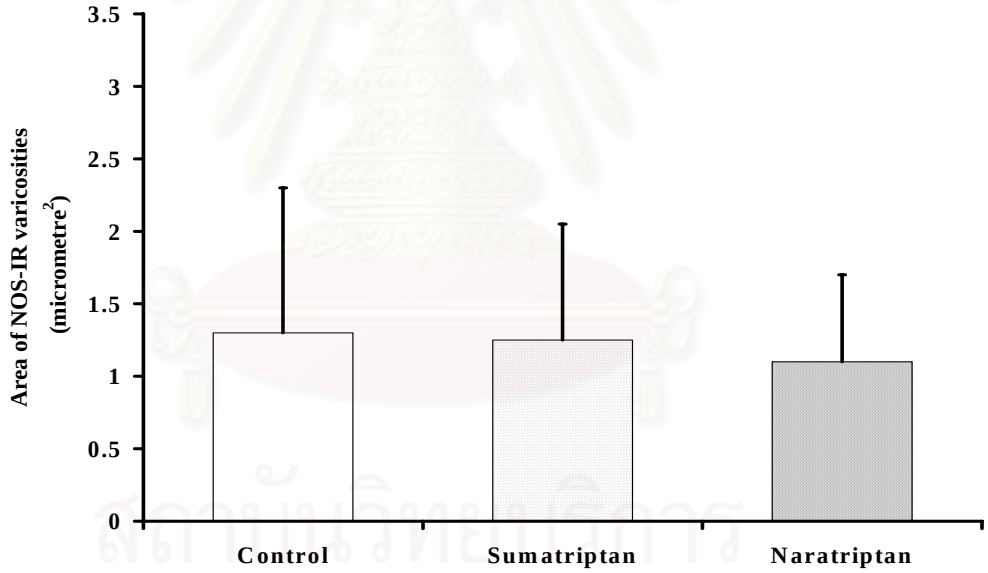
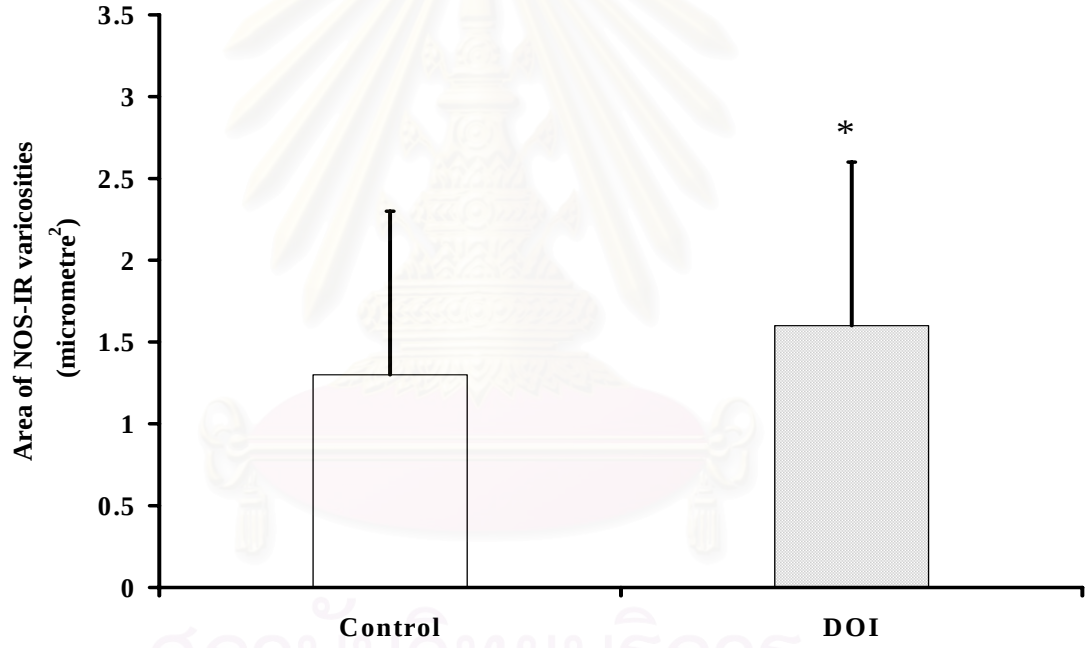


Figure 5.25 *Bar graph showing the mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from control,sumatriptan and naratriptan-treated rats.*

Table 5.13 *The mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from control and DOI-treated rats.*

Group	Mean ± SD of area of NOS-IR varicosities (μm^2)
Control	1.3 ± 1.0
DOI	1.6 ± 1.0 *

* significantly different from control with p<0.01



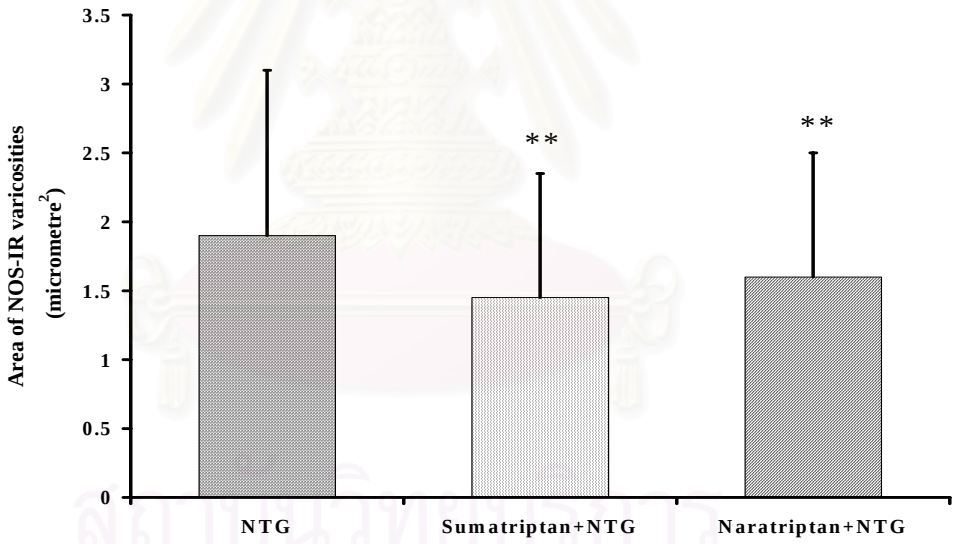
* significantly different from control with p<0.01

Figure 5.26 *Bar graph showing the mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from control and DOI-treated rats.*

Table 5.14 *The mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from NTG, sumatriptan and naratriptan with NTG-treated rats.*

Group	Mean ± SD of area of NOS-IR varicosities (μm^2)
NTG	1.9 ± 1.2
Sumatriptan + NTG	1.45 ± 0.9 **
Naratriptan + NTG	1.6 ± 0.9 **

** significantly different from NTG with p<0.01



** significantly different from NTG with p<0.01

Figure 5.27 *Bar graph showing the mean ± SD of NOS-IR varicosities in superior sagittal sinus obtained from NTG, sumatriptan and naratriptan with NTG-treated rats.*

Table 5.15 *The mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from NTG and DOI with NTG-treated rats.*

Group	Mean ± SD of area of NOS-IR varicosities (μm^2)
NTG	1.9 ± 1.2
DOI + NTG	1.7 ± 1.1

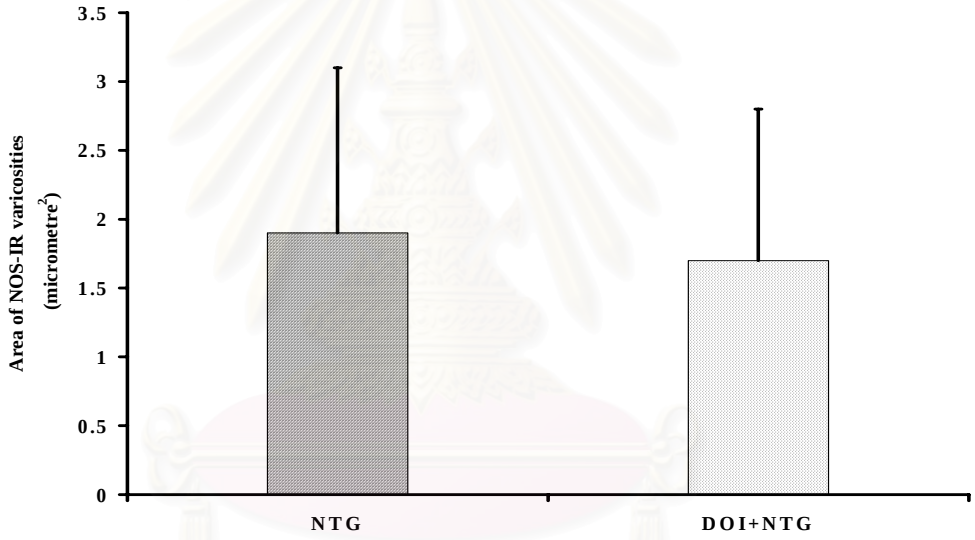


Figure 5.28 *Bar graph showing the mean ± SD of area of NOS-IR varicosities in superior sagittal sinus obtained from NTG and DOI with NTG-treated rats.*

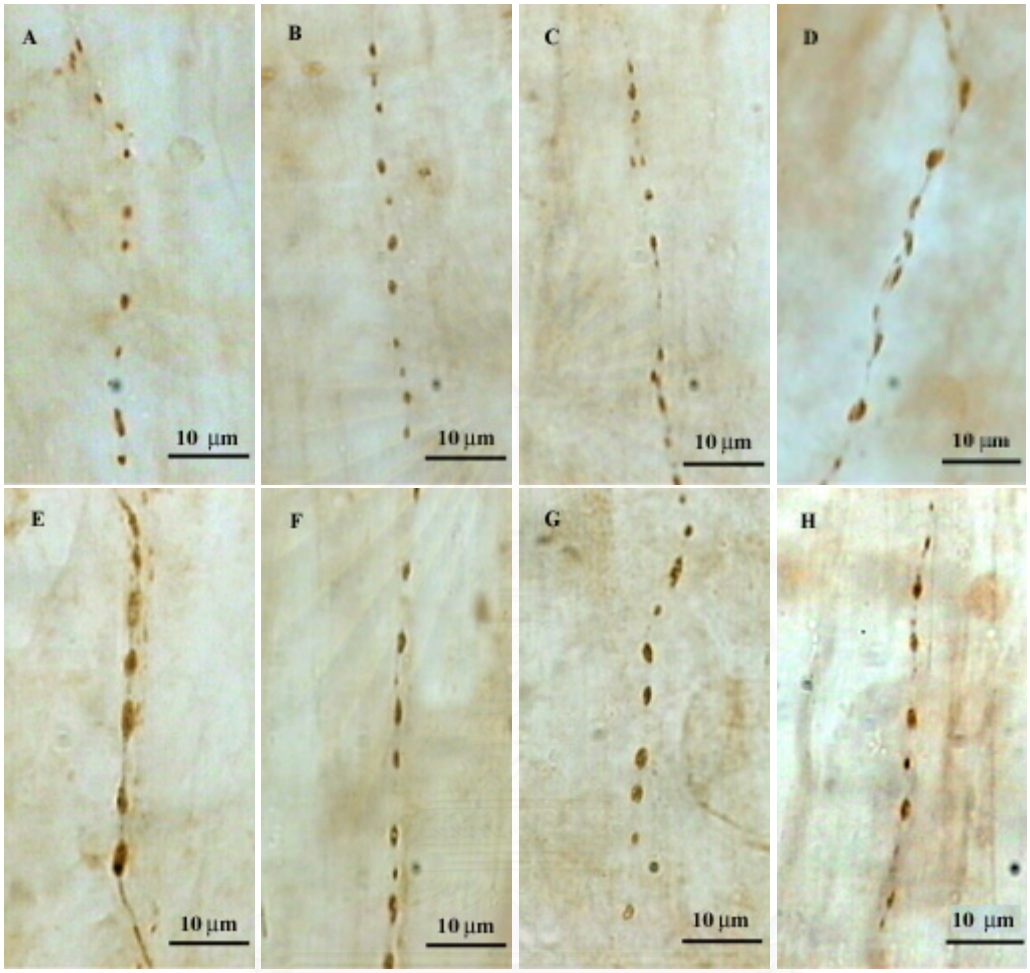


Figure 5.29 *The photomicrograph showing perivascular NOS-immunoreactive nerve fiber on the wall of rat superior sagittal sinus of A) NSS treated, B) sumatriptan-treated, C) naratriptan-treated, D) DOI-treated, E) NTG treated, F) sumatriptan with NTG-treated, G) naratriptan with NTG-treated and H) DOI with NTG-treated rat.*

CHAPTER VI

DISCUSSION

In this study, a series of experiments was conducted to investigate the interaction between two neurotransmitters, namely 5-HT and NO on the trigeminovascular system. These two transmitters are recognised to play significant roles in the process of cerebrovascular nociception based on their vasoactive and pain-modulating properties. In the clinical context, these effects may underlie the development of migraine headache. The effects of different 5-HT receptor subtypes on the cerebrovascular nociceptive system are also addressed in this study.

Effect of NO on trigeminovascular system

Several chemicals orchestrate in the process of cerebral vasodilation. Among these transmitters, NO is of prime importance in the process. This gaseous molecule exerts its vasodilating effect by activating the intracellular cGMP production and minimizing the intracellular calcium. Various structures including endothelial cell, perivascular nerve fibers, intrinsic nNOS positive neurons, etc. are responsible for perivascular NO production. In this experiment, effect of NO on the cerebrovascular system was studied by administration of NTG. NTG was chosen for the study since it can directly liberate NO without the endothelial mechanism. It has been previously demonstrated that exogenous NTG administration led to substantial changes in cerebral vascular system. On the vascular side, NTG can induce marked and prolonged vasodilation. Endothelial cells also undergo morphological changes including mitochondrial swelling, microvillous formation and increase in pinocytosis (Anuntasethakul et al., 1999). It also showed that this NO-induced morphological alteration was enhanced in the 5-HT-depleted state (Srikiatkhachorn et al., 2000). Exposure to NTG also induced expression

of Fos protein in neurons of various areas of brainstem. Interestingly, most of these areas related to the process of craniovascular nociception, e.g. TNC, nucleus of tractus solitarius, nucleus reticularis lateralis, etc (Tassorelli and Joseph., 1995, Srikiatkhachorn et al., 2001).

In this study, the effect of NTG on rCBF was determined by laser Doppler flowmetry. The results showed that administration of NTG induced initial decrease in rCBF, which was following by long-lasting rCBF increase. The initial decline was likely to be secondary to the decrease in systemic blood pressure. The rCBF raised almost immediately after NTG was discontinued and remained increased for at least one hour. This observation is consistent with the previous studies by Anuntasethakul et al (1999) and Srikiatkhachorn et al (2000), which demonstrated the effect of NTG in induction of prolonged pial microvascular dilatation. The phenomenon of NTG-induced prolonged cerebral hyperemia is of interest. It is known that NTG exerts its vasodilating effect by liberating NO. Although NO is very potent vasodilating substance, the duration of its effect is rather limited due to very short half-life of this molecule. The duration of the observed vascular relaxation effect reported herein is well beyond what can be explained by direct effect of NTG alone. Therefore, other mechanisms should be responsible for this process. Besides direct vasodilation, NTG can also exert various physiological effects. Direct application of NTG into brain nuclei causes a prompt increase in the neuronal discharge rate (Tassorelli et al., 1999). This NO-evoked depolarization can lead to other physiological events including the release of vasoactive transmitters. In 1992, Wei et al showed that local application of NTG to pial surface was able to release CGRP from the perivascular nerve plexus (Wei et al., 1992). Thus, NTG can induce prolonged vasodilation by releasing endogenous vasoactive chemicals.

It has been demonstrated in this study that superior sagittal sinus are highly innervated with NOS-IR fiber. Interestingly, it was found that the size

of nNOS-IR perivascular varicosity of the NTG-treated group was greater than the control. This finding is in accord with the previous observation of Knyihar-Csillik and Vecsei (1999) of an increase in beading of the dural NOS-IR nerve fibers after being exposed to NTG. Since NO is a sole active chemical of NTG, it can be concluded that NO is responsible for this structural change.

It is obvious that no definite functional changes can be concluded with confidence based on the morphological finding alone. However, the result of the immunohistochemical study reported here may imply some underlying physiological alterations. For instance, if the size of the varicosity relates to its enzymatic function, the enlargement of nNOS-IR varicosity may reflect an increase in its activity. If this assumption is hold, it can be proposed that exogenous NO can induce prolong vasodilation by activation of the intrinsic NO production. This assumption is in line with the previous findings of Read and his co-workers, which showed that a brief period of NTG infusion resulted in the long-lasting elevation of NO in cortical tissue (Read et al., 1997).

The present study also showed that, at least in part, perivascular nNOS fibers arise from the small- and medium-sized neurons in trigeminal ganglia, which are likely to be nociceptive neurons. Recently, the presence of nNOS-IR neurons has been confirmed in human trigeminal ganglia by Hou et al (2001). Another possible source of perivascular nNOS fiber is parasympathetic ganglia, especially sphenopalatine ganglion. Unfortunately, this structure was not studied in this experiment.

The observation of the NO-evoked nNOS expression in TNC implies that this transmitter is important in the process of cranial nociception. In 1995, Tassorelli and Joseph reported that administration of NTG triggered Fos expression in various brainstem areas, including TNC. Interestingly, it was found that the Fos-IR neurons were in proximity with nNOS-IR neurons and

in some areas both proteins were co-localized in the same neuron (Leong SK et al.,2000). The authours concluded that NTG administration activates a selective group of neurons, which are a source of NO. They also suggested that the NOS synthesizing pathway might be involved at various levels in the central effect of NTG.

Accumulating evidence indicate the role of NO in nociceptive transmission and modulation. According to Wang et al, the numbers of both formalin- and NMDA-evoked FosIR cells in spinal dorsal horn could be reduced by co-injection with the NOS inhibitor at the injured sites (Wang et al., 1999). These results suggest that endogenously generated NO may enhance the initiation of nociceptive inputs of peripheral nociceptors. In addition to its peripheral action, the roles of NO in nociceptive modulation in the central nervous system area also evidenced. For instance, the intrathecal administration of NOS inhibitor was shown to minimize the number of nociception-evoked Fos expression in spinal dorsal horn (Gao and Qiao., 1998). Co-localization of NADPH- diaphorase positive neurons and nociceptive responsive neurons in various brain areas also imply the roles of NO in nociceptive modulation at the supraspinal levels (Rodella et al., 1999). Administration of NO antagonist can reduce the number of Fos expression in the trigeminocervical complex of the cat after stimulation of the superior sagittal sinus (Hoskin et al., 1999). More recently, it was shown that NO was able to potentiate the response of trigeminal neurons. Jones et al (2001) showed that after NTG infusion, responses of trigeminal neurons to electrical stimulation of periorbital cutaneous afferents were potentiated and threshold for activation of these neurons by stimulation of dural afferents was reduced. They also found that pretreatment with NTG could enhance the response of trigeminal neurones to chemical irritation as evident by an increase in the number of Fos-IR in trigeminal caudalis. A possible explanation of the facilitating effect of NO on the central nociception is an enhancement of

glutamate release resembling the process of long-term potentiation and the development of central sensitization.

Based on the above evidence including the findings from the present study, two mechanisms of NTG in cerebrovascular nociception can be proposed. First, excessive dose of NTG can induce long-lasting vasodilation by activating the endogenous NO production. Second, NTG can enhance nNOS expression in the TNC and trigeminal ganglia might facilitate the process of nociception.

5-HT receptors and modulation of trigeminovascular system

It has long been held that 5-HT plays a pivotal role in migraine pathogenesis. This transmitter exerts it various physiological effects via its vast diversity of receptor subtypes. At least 15 subtypes of 5HT receptors have been characterized. The effects of two 5-HT subtypes, namely 5-$HT_{1B/1D}$ and 5-$HT_{2A/2C}$ receptors, were determined in this study. These two subtypes were chosen for investigation since they are abundant in the trigeminovascular system. Moreover, a number of anti-migraine compound act on these receptors.

In this series of experiments, effect of 5-HT receptor agonists on cerebrovascular nociception were determined in two conditions, resting and NTG-evoked states. The interaction between NO and 5-HT has been previously addressed. In 2000, Srikiatkhachorn et al showed that the degree of pial and cerebral microvascular response to NTG was greater in the 5-HT-depleted animals. The authours proposed that the decreased serotonergic activity might increase the sensitivity of cranial vascular to NO. Supersensitivity to NO has been proposed to be a mechanism of migraine pathogenesis (Thomsen and Olesen., 1997).

Effect of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors

The present results showed that administration of 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor agonists did not alter the rCBF or MABP in the resting condition. Interestingly, it was found that pretreatment with 5-HT$_{1B/1D}$ agonist, sumatriptan, could shorten the duration of NTG-evoked cerebral hyperemia without changing the magnitude of the initial hyperemic phase. In this group, the rCBF became normalized at minute 25 post-NTG-infusion, whilst the rCBF remained increased in the NSS-pretreated group. Unfortunately, this observed change could not reach the level of statistical significance. This is likely to be the result of poor statistical power since the number of samples in each group is rather small. The study with larger number of samples is needed to confirm this finding.

The changes in rCBF discussed above were in accord with the immunohistochemical findings. In the morphological study, it was demonstrated that naratriptan and sumatriptan pretreatment were able to block the effect of NTG in enlarging the perivascular nNOS-IR varicose. The pattern of rCBF and morphological changes imply that activation of 5-HT$_{1B/1D}$ receptors can shorten the duration of NTG-evoked cerebral hyperemia by inhibiting the process endogenous NO production. The effect of sumatriptan and naratriptan in minimizing the degree of NO-evoked nNOS expression was also evident in the trigeminal ganglia and TNC.

Several mechanisms are proposed to explain the pharmacological efficacy of triptan compounds. This includes induction of vasoconstriction, inhibition of algogenic peptide release and stabilizing the thalamic projection neurons. Recently, Read et al (1999) showed that administration of NTG led to increased NO and decreased superoxide concentrations. This reverse

relationship was due to the use of superoxide in the process of peroxynitrite formation. Interestingly, sumatriptan can stabilize the level of superoxide. The authours proposed that the mechanisms of this action of sumatriptan may include modulation of cell redox state, NO scavenging or direct manipulation of superoxide release (Read et al., 1999). The present study indicates another possible effect of the compounds of this family in modulation of NO system. At periphery, activation of 5-$HT_{1B/1D}$ receptors may prevent prolonged vasodilation by blocking the endogenous NO production. In the central synapses, activation of this receptor can prevent the nociception-evoked nNOS expression in TNC. This conclusion is in line with the observation that 5-$HT_{1B/1D}$ agonists can abolish the NMDA-evoked enhancement of NOS activity and cGMP concentration (Stepien et al., 1999). Since the nNOS-IR neurons can facilitate the nociceptive processing, decrease the nNOS expression will stabilize the thalamic projection cells and decrease their firing rate.

Effect of 5-$HT_{2A/2C}$ receptors

Unlike the effect of 5-$HT_{1B/1D}$ receptors, activation of $HT_{2A/2C}$ receptors leads to a rise in both systemic blood pressure and rCBF. This type of receptor belongs to G protein-coupled superfamily. The activation of this receptor facilitates the process of phosphoinositol hydrolysis. As a result, the level of cytoplasmic inositol triphosphate rises. Binding of this second messenger molecule to its receptor on endoplasmic reticulum will lead to the release of calcium from its intracellular store. In vascular wall, rising of the intracellular calcium will trigger the phosphorylation-dephosphorylation of motor molecule resulting in smooth muscle contraction. This vascular constriction effect will raise the total peripheral resistance and increase systemic blood pressure.

Although the activation of this receptor can increase the blood pressure via the above mechanism, the increase in rCBF observed in this experiment is unlikely to be secondary to an increase in the perfusion pressure. It is well accepted that cerebral circulation is strictly controlled by the process of autoregulation. This process stabilizes the CBF and does not allow the CBF to fluctuate according to the systemic blood pressure. In brain, the lower and upper limit of the autoregulatory plateau have been determined as approximately 50-60 and 150-160 mmHg, respectively. Thus, once the limits of autoregulation are reached, CBF will increase or decrease passively with elevation or reductions in perfusion pressure (Chillon and Baumbach, 1997). An increase in MABP in the DOI-treated animals that observed in this study is still in the range of autoregulation. Thus it cannot alter the rCBF.

The immunohistochemical study showed that administration of DOI was able to enlarge the perivascular nNOS-IR varicose as well as the number of nNOS-IR cells in trigeminal ganglia. These findings resemble those observed in the NTG-treated group. It is known that activity of nNOS is calcium dependent. An increase of intracellular calcium via phosphoinositol hydrolysis, which is secondary to $HT_{2A/2C}$ receptor activation may activate this enzyme and facilitate the NO synthesis. Therefore, it can be proposed that activation of 5-$HT_{2A/2C}$ receptor will increase the rCBF by activating the perivascular nNOS fiber, hence increase the NO production.

Besides the changes in peripheral tissue, administration of DOI also increased the nNOS expression in TNC neurons. This finding raises the possibility that activation of 5-$HT_{2A/2C}$ receptor may facilitate the nociceptive process and increases the susceptibility to pain. It has been observed that 5-$HT_{2A/2C}$ receptor may have nociceptive potentiating effect by enhancing the release of substance P from the primary afferent (Eide and Hole., 1993). Supraspinal mechanism of this 5-$HT_{2A/2C}$ -induced nociceptive facilitation has also been suggested (Alheider, 1991). Since hydrolysis of phosphoinositol is a

transduction cascade of this receptor type, occupying of these receptors will activate the release of calcium from its intracellular store. A rising in the intracellular calcium is an important step in the development of long-term potentiation and central sensitization. Interestingly, many migraine prophylactic drugs, i.e. cyproheptadine, pizotifen, etc., act by antagonizing the effect of this receptor type. Down-regulation of this receptor has been proposed to underlie the anti-nociceptive effect of non-narcotic analgesics including paracetamol (Srikiatkhachorn et al., 1999).

The above evidences suggest that activation of $HT_{2A/2C}$ receptor may increase the pain susceptibility. The finding of $HT_{2A/2C}$ receptor agonist-induced nNOS expression in TNC further elaborates the nociceptive facilitating effect of this receptor. In the context of vascular nociception, activation of this receptor may induce vasodilation, sensitizing the perivascular nociceptor and facilitating the thalamic projection neurons in the TNC.

The implication to migraine pathogenesis

This study demonstrates the complex relationship between NO and 5-HT in control of cerebrovascular nociception. This relationship is further complicated by the observation that different 5-HT receptor subtypes affect the trigeminovascular system differently. It can be concluded from the present study that activation of 5-$HT_{1B/1D}$ receptors will antagonize the effect of NO in raising the CBF and activation of intrinsic nNOS system. These effects are reverse in the case of 5-$HT_{2A/2C}$ receptors. Application of 5-$HT_{2A/2C}$ receptor agonist induces cerebral hyperemia and enhances the expression of nNOS enzyme in various structures.

The above conclusions are well aligned with the clinical information of migraine. It has been well demonstrated that 5-$HT_{1B/1D}$ receptor agonists

especially the triptan family is effective in aborting the attack of migraine. On the contrary, migraine attacks can be prevented by blocking the 5-$HT_{2A/2C}$ receptors. These pharmacological effects imply the anti-migraine property of 5-$HT_{1B/1D}$ receptor and pro-nociceptive property of 5-$HT_{2A/2C}$ receptors. An increase in maximum number of binding sites of the 5-$HT_{2A/2C}$ receptors has been demonstrated in platelets taken from migraine patients with analgesics rebound headache (Srikiatkhachorn et al 1998). In this condition, the feature of headache evolves from episodic headache as seen in usual migraine to daily headache. The up-regulation of 5-$HT_{2A/2C}$ receptors has been proposed for this evolution. This phenomenon also confirms the pro-nociceptive activity of the 5-$HT_{2A/2C}$ receptor.

Although 5-HT can bind to both 5-$HT_{1B/1D}$ and 5-$HT_{2A/2C}$ receptors, its affinities to these receptor are different. This transmitter binds to 5-$HT_{1B/1D}$ receptor with higher affinities. At low concentration, 5-HT can bind to 5-$HT_{1B/1D}$ receptor more readily and will occupy the 5-$HT_{2A/2C}$ receptors at the higher concentration. Therefore, it suggests that at low or physiologic concentration, 5-HT acts as anti-migraine chemical. On the contrary, high or pathological concentration of 5-HT will facilitate the nociception as well as increase the CBF. It is proposed that 5-HT is released during the initial phase of migraine attack (Anthony et al 1969). This released 5-HT may activate the $HT_{2A/2C}$ receptors, resulting in nociceptive facilitation. This conclusion agrees with previous hypothesis by Fozard, which stated that release of 5-HT during migraine attacks might increase NO production via the activation of $HT_{2A/2C}$ receptor (Fozard, 1999).

CHAPTER VII

CONCLUSION

This study investigated the effects of serotonin receptor agonists on the cerebrovascular nociception. Sumatriptan and naratriptan were used as 5-$HT_{1B/1D}$ receptor agonist and DOI was used as 5-$HT_{2A/2C}$ receptor agonist.

The results showed that neither sumatriptan nor naratriptan alter the basal rCBF. However, in animal pretreated with both of them could minimized the degree of NO-induced hyperemia in cerebral cortical tissues. The immunohistochemical study showed that exposure to NO-donating agent could induce expression of nNOS enzyme in various structures of the trigeminovascular pathway (trigeminal nucleus caudalis, trigeminal ganglion and perivascular nerve fiber). Pretreatment with 5-$HT_{1B/1D}$ agonist, sumatriptan, could decrease the NO-evoked nNOS expression in all area. On the other hand, injection of DOI could significantly increase the basal rCBF. The administration of DOI could induce nNOS-immunoreactivity in all structures of trigeminovascular pathway but could not alter the effects of NO-evoked nNOS expression.

Based on these findings, we suggest that activation of 5-$HT_{2A/2C}$ receptor results in long-lasting vasodilatation by activating the endogeneous nNOS system.These effects resemble the effect of NTG. However, activation of 5-$HT_{1B/1D}$ produced the opposite effects. Administration of agonists to these receptor can attenuate the effects of NTG-induced activation of nNOS system. These results provide the further evidence in supporting the roles of 5-HT and NO in control of trigeminovascular nociception.

REFERENCES

Adham IM et al. Cloning and characterisation of a recombinant guinea pig 5-HT_{1F} receptor (abstract). **Amsoc Neurosci** 1996;22-6.

Aimi Y, Fujimura M, Vincent SR, Kimura H. Localization of NADPH-diaphorasc-containing neurons in sensory ganglia of the rat. **J Comp Neurol** 1991; 306: 382-92.

Alhaider AA. Antinociceptive effects of ketanserin in mice: involvement of supraspinal 5-HT_2 receptors in nociceptive transmission. **Brain Res** 1991; 543: 335-40.

Andres KH, Von DM, Muszynski K, Schmidt RF. Nerve fibres and their terminals of the duramater encephali of the rat. **Anat Embryol** 1987; 175: 289-301.

Anthony M, Hinterberger H, Lance JW. The possible relationship of serotonin to the migraine syndrome. In: Friendman AP, editor**. Research and Clinical Studies in Headache**. New York: S. Karger, 1969: 29-59.

Anthony M., Hinterberger H, Lance J.W. Plasma serotonin in migraine and stress. **Arch. Neurol** 1967; 16: 544-52.

Anuntasethakul T, Srikiatkhachorn A, Maneesri S, Patumraj S, Kasantikul V. Ultrastructural changes in endothelial cells of cerebral microvessels after exposure to nitric oxide donor. **Neuropathology** 1999; 19: 249-58.

Arbab MD, Wiklund L, Svendgaard NA. Origin and distribution of cerebral vascular innervation from superior cervical, trigeminal and spinal ganglia investigated with retrograde and anterograde WGA-HRP tracing in the rat. **Neuroscience** 1986; 19: 695-708.

Ariano MA. Distribution of components of the guanosine 3', 5'-phosphate system in rat caudate-putamen. **Neuroscience** 1983; 10: 707-23.

Axelsson KL, Wikberg JE, Anderson RG. Relationship between nitroglycerin, cyclic GMP and relaxation of vascular smooth muscle. **Life Sci** 1979; 24: 1779-89.

Baringa M. Is nitric oxide the 'retrograde messenger'. **Science** 1991; 254: 1296-323.

Basbaum AI, Jessel TM. The Perception of Pain. In Kendel ER, Schwartz JH, Jessel TM, editors. **Principles of neuroscience**. New York: McGraw-Hill,2000:472-90.

Bates D, Ashford E., Dawson R, Ensink F.-B.M, Gilhus NE, Olesen J, et al. Subcutaneous sumatriptan during the migraine aura. **Neurology** 1994; 44: 1587-92.

Bauer MB, Murphy S, Gebhart GF. Capsaicin stimulates cGMP via nitric oxide in dorsal root ganglion cells (abstract). **Soc Neurosci** 1992; 18: 689-95.

Bax WA, Heuven-Nolsen DV, Simoons ML, Saxena PR. 5-Hydroxytryptamine induced contractions of the human isolated sphenous vein: involvement of 5-HT_2 and 5-HT_{1D}- like receptors and a comparison with grafted brains. **Naunyn-Schmiedeberg's Arch Pharmacol** 1992; 345: 500-8.

Baxter G, Kennett G, Blaney F, Blackburn T. 5-HT_2 receptor subtypes: a family re-united? **Trends Pharmacol Sci** 1995; 16 (3): 105-10.

Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. **Pain** 1988; 33: 87-107.

Bereiter DA, Benetti AP. Excitatory amino release within spinal trigeminal nucleus after mustard oil injection into the temporomandibular joint region of the rat. **Pain** 1996; 67: 451-59.

Berger RJ, Zuccarello M, Keller JT. Nitric oxide synthase immunoreactivity in the rat dura mater. **Neuro Report** 1994; 5: 519-21.

Boadle-Biber MC. Regulation of serotonin synthesis. **Pro Biophys molec Biol** 1993; 60: 1-15.

Bockaert J et al. 5-HT_4 receptors: potential therapeutic implications in neurology and psychiatry. **CNS Drugs** 1994; 16: 30-7.

Bodelsson M, Törnebrandt K, Arneklo-Nobin B. Endothelial relaxing 5-hydroxytryptamine receptors in the rat jugular vein: similarity with the 5-hydroxytryptamine$_{1C}$ receptor. **J Pharmacol exp Th**er 1993; 264: 709-16.

Bou J, Domenech T, Gras J, Beleta J, Llenas J and Fernandez AG. a novel antimigraine agent**. Cephalalgia** 1997; 17: 421-2.

Bredt DS, Glatt CE, Hwang PM, Fotuhi M, Dawson TM, Synder SH. Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. **Neuron** 1991; 7: 615-24.

Brewerton TD, Murphy DL, Lesem MD, Brandt HA, Jimerson DC. Headache responses following m-chlorophenylpiperazine in bulimics and controls. **Headache** 1992; 32: 217-22.

Bult H, Boeckxstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, Herman AG Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. **Nature** 1990; 345: 346-7.

Burstein R, Yamamura H, Malick A, and Strassman AM. Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. **J. Neurophysiol**. 1998; 79: 964-82.

Busse R, Mülsch A. Induction of nitric oxide synthase by cytokines in vascular smooth muscle cells. **FEBS *Lett*** 1990; 275: 87-90.

Buzzi MG and Moskowitz MA The antimigraine drug sumatriptan (GR43175) selectively blocks neurogenic plasma extravasation from blood vessels in dura mater. **Br. J. Pharmacol**. 1990; 99: 202-6.

Buzzi MG, Moskowitz MA, Shimizu T, and Health HH. Dihydroergotamine and sumatriptan attenuate levels of cGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. **Neuropharmacol**. 1991; 30: 1193-200.

Buzzi MG, Sakas DE, and Moskowitz MA. Indomethacin and acetylsalicylic acid block neurogenic plasma protein extravasation in rat dura mater. **Eur.J. Pharmacol**. 1989; 165: 251-8.

Cameron AA, Leah JD, Snow PJ. The coexistence of neuropeptides in feline sensory neurons. **Neuroscience** 1988; 27: 969-79.

Carignani C, Ugolini A, Pinnola V, Belardetti F, Trist DG and Corsi M. NMDA receptor subunit characterization of the glycine antagonist GV196771A and its action on the spinal cord wind-up. **Naunyn-Schmiedeberg's Archives of Pharmacolgy** 1998; 358 (151): 1119.

Carson MA et al. The 5-HT_{5A} serotonin receptor is expressed predominantly on astrocytes witthin the developing and adult rat CNS (abstract). **Am Soc Neurosci** 1995; 21: 728-32.

Cesselin F, Le Bars DL, Bourgoin S, Artaud F, Gozlan H, Clot Am et al. Spontaneous and evoked release of methionine-enkephalin like material from the rat spinal cord in vivo. **Brain Res** 1985; 339: 305-13.

Chol DS and Maroteaux L. Immunohistochemical localization of serotonin 5-HT_{2B} receptor in mounse gut, cardiovascular system and brain. **FEBS *lett*** 1996; 391(1-2): 45-51.

Cirino G, Cicala C, Sorrentino L, Regoli D. Effect of bradykinin antagonists, N^G-monomethyl-L-arginine and L-N^G nitroarginine on phospholipase A_2 induced oedema in rat paw. **Gen Pharmacol** 1991; 22: 801-4.

Clark D, Hough H, Wolff HG. Experimental studies on headache: observations on headache produced by histamine. **Arch Neurol Psychiatry** 1936; 35: 1054-69.

Classey JD, Knight YE and Goadsby PJ. MK-801 reduces Fos-like immunoreactivity in trigeminocervical complex of cat. **Cephalalgia.** 1999; 19: 394-401.

Clitherow JW, Scopes DI, Skingle M, Jordan CC, Feniuk W, Campbell IB et al. Evolution of a novel series of [(N,N-dimethylmino) propyl] and peperazinylbenzanilides as the first selective 5-HT_{1D} antagonists. **J. Medicinal Chemistry**.1994; 37: 2253-7.

Connor HE, Stubbs CM, Feniuk W, and Humphrey PPA. Effect of sumatriptan, a selective 5-HT_1-like receptor agonist, on pial vessel diameter in anaesthetised cats. **J. Cereb. Blood Flow Metabol** 1992; 12: 514-9.

Cox DA, Cohen ML. 5-HT_{2B} receptor signaling in the rat stomach fundus: dependence on calcium influx, calcium release and protein kinase C. Behav. **Brain Res** 1996; 73 (1-2): 289-92.

Cumberbatch M.J, Hill RG, and Hargreaves RJ. Rizatriptan has central antinociceptive effects against durally evoked responses. **Eur. J. Pharmacol.** 1997; 328: 37-40.

Cumberbatch MJ, Williamson DJ, Mason GS, Hill RG, and Harraeaves RJ. Dural vasodilatation causes a sensitization of rat caudal trigeminal neurones in vivo that is blocked by a 5-$HT_{1B/1D}$ agonist. **Br.J. Pharmacol.** 1999; 126: 1478-86.

Cumberbatch MJ, Hill RG, and Hargreaves RJ. Differential effects of the $5HT_{1B/1D}$ receptor agonist naratriptan on trigeminal versus spinal nociceptive responses. **Cephalalgia** 1998a; 18: 659-64.

Cumberbatch MJ, Hill RG, and Hargreaves RJ. The effects of 5-$HT1_B$ and 5-$HT1_D$ receptor agonists on trigeminal nociceptive neurotransmission in anaesthetized rats. **Eur J Pharmacol** 1998b; 362: 43-6.

Curran D, Hinterberger H, and Lance JW. Total plasma serotonin, 5-hydroxyindoleacetic acid and p-hydroxy-m-methoxy-mandelic acid excretion in normal and migrainous subjects. **Brain** 1965; 88: 997-1010.

Dahl A, RusseD, Nyberg-Hansen R, Rootwell K. Effect of nitroglycerin on cerebral circulation measured by transcranial Doppler and SPECT. **Stroke** 1989; 20: 1733-6.

Davis KD and Dostrovsky JO. Activation of trigeminal brainstem nociceptive neurons by dural artery stimulation. **Pain** 1986; 25: 395-401.

Davis KD and Dostrovsky JO. Responses of feline trigeminal spinal tract nucleus neurons to stimulation of the middle meningeal artery and sagittal sinus. **J. Neurophysiol.** 1988; 59: 648-66.

Dawson TM, Dawson VL, Synder SH. A novel neuronal messenger molecule in brain: the free radical, nitric oxide. **Ann Neurol** 1992; 32: 297-311.

de Vrics P, Apaydin S, Villalon CM, Heiligers JP and Saxcna, PR. Interactions of GR127935 a 5-HT (1B/1D) receptor ligand with functional 5-HT receptors. **Naunyn Schmiedeberg's Arch. Pharmacol**. 1997; 355 (4): 423-30.

Den Boer MO, Villalon CM, and Saxena PR. 5-HT_1-like receptors mediate changes in porcine carotid hemodynamics are 5-HT_{1D} receptors involved? **Naunyn-Schmiedeberg's Arch. Pharmacol.** 1992; 345: 509-15.

Diener HC. Substance-P antagonist RPR100893-201 is not effective in human migraine attacks. In: Olesen, J., Tfelt-hansen, P, editors. **Proceedings of the VI th International Headache Seminar.** Lippincott-Raven, New York. 1996.

Dimitriadou V, Buzzi MG, Theoharides TC, and Moskowitz, MA. Ultrastructural evidence for neurogenically mediated changes in bloodvessels of the rat dura mater and tongue following antidromic trigeminal stimulation. **Neuroscience** 1992; 48: 187-203.

Doenicke A, Siegel E., Hadoke M., and Perrin V.L. Initial clinical study of AH25086B (5-HT1-like agonist) in the acute treatment of migraine. **Cephalalgia** 1987; 7: 437-8.

Doi-Saika M, Tokunaga A, Senba E. Intradermal 5-HT induces Fos expression in rat dorsal horn neurons not via 5-HT_3 but via 5-HT_{2A} receptors (abstract). **Neuroscience Res** 1997; 29(2): 143-9.

Doyle MP, Hoekstra JW. Oxidation of nitrogen oxides by bound dioxygen in hemoproteins. **J Inorg Biochem** 1981; 14: 351-8.

Draisci G, Iadarola MJ. Temporal analysis of increase in c-fos, preprodynorphin and preproenkephalin mRNAs in rat spinal cord. **Molec Brain Res** 1989; 6: 31-7.

Drummond PD, Gonski A and Lance JW. Facial flushing after thermocoagulation of the gasserian ganglion**. J. Neurol. Neurosurg. Psychiatry** 1983; 46: 611-6.

Duarte IDG, Lorenzetti BB, Ferreira SH. Peripheral anal-gesia and activation of the nitric oxide-cyclic GMP pathway. **Eur J Pharmacol** 1990; 186: 289-93.

Dubner R, Ruda MA. Activity-dependent neuronal plasticity following tissue injury and inflammation. **Trends Neurol Sci** 1992; 15: 96-103.

Dun NJ, Dun SL, Forsermann U, Tseng LF. Nitric oxide synthase immunoreactivity in rat spinal cord. **Neurosci *Lett*** 1992; 147: 217-20.

Duxon L et al. Evidence for the expression of 5-HT_{2B} receptor protein in the rat central nervous system. **Neuroscience** 1997; 76: 201-6.

Edvinsson L, Goadsby PJ. Neuropeptides in migraine and cluster headache. **Cephalalgia** 1994; 14: 320-8.

Edvinsson L, Goadsby PJ. Neuropeptides in headache. **Eur J Neurology** 1998; 5: 329-41.

Eglen RM, Jasper JR, Chang DJ, Martin GR. The 5-HT_7, receptor: Orphan found. **Trends Pharmacol Sci** 1997; 18 (4): 104-8.

Eide PK, Hole K: Interactions between serotonin and substance P in the spinal regulation of nociception. **Brain Res** 1991; 550: 225-30.

Fanciullacci M, Alessandri M, Figini M, Geppetti P, and Michelacci S. Increases in plasma calcitonin gene-related peptide from extracerebral circulation during nitroglycerin-induced cluster headache attack. **Pain** 1995; 60: 119-23.

Fang JWV, Lin Q, Willis WD. For expression is induced by increased nitric oxide release in rat spinal cora dorsal horn. **Neuroscience** 1999; 96 (2): 351-7.

Feelisch M, Noack EA. Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. **Eur J Pharmacol** 1987; 139: 19-30.

Feelisch M. Biotransformation to nitric oxideof organic nitrates in comparison to other nitrovasodilators. **Eur Heart J** 1993; 14 Suppl 1: 123-32.

Felley-Bosco E, Ambs S, Lowenstein CS, Keefer LK, Haris CC. Constitutive expression of inducible nitric oxide synthase in human bronchial epithelial cells induces c-fos and stimulates c-GMP pathways. **Am J Respir Cell Mol Biol** 1991; 11: 159-64.

Ferrari MD, Saxena PR. On serotonin and migraine: a clinical and pharmacological review. **Cephalalgia** 1993; 13: 151-65.

Fletcher A, Cliffe IA, Dourish CT. Silent 5-HT_{1A} receptor antagonists:utility as research tools and therapeutic agents. **Trends Pharmacol Sci** 1993; 14 (12): 41-8.

Foguet M, Hoyer D, Pardo LA, Parekh A, Kluxen FW, Kalkman HO, Stübbert H. Cloning and functional characterization of the rat stomach fundus serotonin receptor. **EMBO J** 1992; 11: 3481-7.

Forrari MD, Odink J, Tapparelli C, Van Kempen GMJ, Pennings EJM, Bruyn GW. Serotonin metabolism in migraine. **Neurology** 1989; 39: 1239-42.

Forstermann U, Schmidt HHW, Pollock JS, Sheng H, Mitchell JA, Warner TD, Nakane M, Murad F. Isoforms of nitric oxide synthase. Characterization and purification from different cell types. **Biochem Pharmacol** 1991; 10: 1849-57.

Fozard JR. Serotonin, migraine and platelets**. Prog in Pharmacol** 1982; 414: 135-46.

Fozard JR. 5-HT in migraine: evidence from 5-HT receptor antagonists for a neuronal aetiology. In: Sandler M, Collins GM ,editors. **Migraine: a spectrum of ideas**. Oxford: Oxford University Press , 1990: 128-46.

Fozard JR. $5HT_{1C}$ receptor agonism as an initiating event in migraine. In: Olesen J, Saxena PR, editors. **5-Hydroxytryptamine mechanisms in primary headache.** New York Raven Press: Raven Press, 1992: 200-12.

Fozard JR.. The 5-hydroxytryptamine-nitric oxide connection: the key link in the initiation of migraine? **Arch Int Pharmacodyn Ther** 1995;329:111-9.

Fozard JR, Gray JA. $5HT_{1C}$ receptor activation: a key step in the initiation of migraine. **Trends Pharmacol Sci** 1989; 10: 307-9.

Fozard JR, Kalkman HO. 5-Hydroxytryptamine (5-HT) and the initiation of migraine: new perspectives. **Naunyn-Schmiedeberg's Arch Pharmacol** 1994; 350: 225-9.

Friberg L, Olesen J, Iversen HK, Sperling B. Migraine pain associated with middle cerebral artery dilatation: Reversal by sumatriptan. **Lancet** 1991; 338: 13-7.

Fujiwara M, Usui H, Kurahashi K, Jino H, Shirahasa H, Mekata F. Endothelium dependent contraction produced by acetylcholine and relaxation produced by histamine in monkey basilar arteries. **J Cardiovasc Pharmacol** 1992; 20: 114-6.

Gallai V, Sarchielli P, Floridi A, Franceschini M, Codini M, Trequattrini A, and Palumbo R. Vasoactive peptides levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally. **Cephalalgia** 1995;15: 384-90.

Gao WC, Qiao JT. Nitric oxide contributes to both spinal nociceptive transmission and its descending inhibition in rats: an immunocytochemical study. **Neurosci L*ett*** 1998;240:143-6.

Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intracellular messenger in the brain. **Nature** 1955; 326: 385-7.

Gerald C, Adham N, Kao HT, Olesen MA, Laz TM, Schechter LE, et al. The 5-HT_4 receptor: molecula5r cloning and pharmacological characterisation of two splice variants. **EMBO J** 1995; 14: 2806-15.

Glusa E, Richter M. Endothelium-dependent relaxation of porcine pulmonary arteries via 5-HT_{1C}-like receptors**. Naunyn-Schmiedegberg's Arch Pharmacol** 1993; 347: 471-7.

Goadsby PJ. Current concepts of the pathophysiology of migraine. **Neurol Clin** 1997; 15 (1): 27-41.

Goadsby PJ. 5-$HT_{1B/1D}$ agonists in migraine comparative pharmacology and its therapeutic implications. **CNS Drugs** 1998a; 10: 271-86.

Goadsby PJ. The pharmacology of headache. **Prog in Neurobio** 2000; 62: 509-25.

Goadsby PJ and Classey JD. Blood flow responses in trigemi-nocervical complex involve glutamate. **Cephalalgia** 1999; 19: 315.

Goadsby PJ and Duckworth. JW. Effect of stimulation of trigeminal ganglion on regional cerebral blood flow in cats. **Am. J. Physiol**. 1987; 253: R270-R400.

Goadsby PJ and Edvinsson L. The trigeminovascular system and migraine: studies characterising cerebrovascular and neuropeptide changes seen in man and cat. **Ann. Neurol**. 1993; 33: 48-56.

Goadsby PJ and Edvinsson L. Human in vivo evidence for trigeminovascular activation in cluster headache. **Brain**. 1994a; 117: 427-34.

Goadsby PJ, Edvinsson L. Peripheral and central trigeminovascular activation in cat is blocked by the serotonin (5HT)-1D receptor agonist 311C90. **Headache** 1994b; 34: 394.

Goadsby PJ and Edvinsson L. Neuropeptide changes in a case of chronic paroxysmal hemicrania-evidence for trigemino-parasympathetic activation. **Cephalalgia** 1996; 16: 448-50.

Goadsby PJ and Edvinsson L. Regulationg of cerebral blood flow by the parasympathetic nervous system. In: Wclch KMA, Caplan L, Rcis DJ, Weir b, Siesjo B, editors. **Primer of Cerebrovascular Diseases**. Academic Press, San Diego, 1997; 63-6.

Goadsby PJ and Edvinsson L. Peripheral and central trigeminovascular activation in cat is blocked by the serotonin (5-HT)-1D receptor agonist 311C90. **Headache** 1999; 34: 394-9.

Goadsby PJ, Edvinsson L, and Ekman R. Release of vasoactive peotides in the extracerebral circulation of man and the cat during activation of the trigeminovascular system. **Ann Neurol** 1988; 23: 193-6.

Goadsby PJ, Edvinsson L, and Ekman R. Vasoactive peotide release in the extracerebral circulation of humans during migraine headache**. Ann. Neurol**. 1990; 28: 183-7.

Goadsby PJ, Edvinsson L, Ekman R. Cutaneous stimulation leading to facial flushing and release of calcitonin gene-related peptide. **Caphalalgia** 1992; 12: 53-6.

Goadsby PJ, Gundlach AL. Localization of [^{3}H] dihydroer-oergotamine binding sites in the cat central nervous system relevance to migraine. **Ann. Neurol**. 1991; 29: 91-4.

Goadsby PJ, Hoskin KL. Inhibition of trigeminal neurons by intravenous administration of the serotonin (5HT)-1-D receptor agonist zolmitriptan (311C90): are brain stem sites a therapeutic target in migraine? **Pain** 1996; 67: 355-9.

Goadsby PJ, Hoskin KL. The distribution of trigeminovascular afferents in the non-human primate brain macaca memestrina: a c-fos immunocytochemical study. **Journal of Anatomy** 1997; 190: 367-75.

Goadsby PJ, Hoskin KL. Serotonin inhibits trigeminal nucleus activity evoked by craniovascular stimulation through a 5-$HT_{1B/1D}$ receptor: a central action in migraine? **Ann. Neurol.** 1998; 43: 711-718.

Goadsby PJ, Hoskin KL. Differential effects of low dose CP122,288 and eletriptan on Fos expression due to stimulation of the superior sagittal sinus in the cat. **Pain** 1999; 82: 15-22.

Goadsby PJ, Kaube H, Hoskin KL. Nitric oxide synthesis couples cerebral blood flow and metabolism. **Brain Res** 1992; 595: 167-70.

Goadsby PJ, Knight YE. Inhibition of trigeminal neurones after intravenous administration of naratriptan through an action at 5-hydroxytry ptamine (5-$HT_{1B/1D}$) receptors. **Br J Pharmacol** 1997a; 122: 918-22.

Goadsby PJ, Knight YE. Direct evidence for central sites of action of zolmitriptan (311C90): An autoradiographic study in cat. **Cephalalgia** 1997b; 17: 153-8.

Goadsby PJ, Knight YE and Hoskin KL. Processing of occipital pain in the caudal trigeminal nucleus: refered pain in primary headache. **Cephalalgia** 1997; 17: 381.

Goadsby, PJ, Lipton RB A review of paroxysmal hemicraniasm SYBCT syndrome and other short-lasting headaches with autonomic features, including new cases. **Brain** 1997; 120: 193-209.

Goadsby PJ, Olesen J. Diagnosis and management of migraine. **BMJ** 1996; 312: 1279-82.

Goadsby PJ, Zagami AS. Stimulation of the superior sagittal sinus increases metabolic activity and blood flow in certain regions of the brainstem and upper cervical spinal cord of the cat. **Brain** 1991; 114: 1001-11.

Goldstein DJ, Offen WW, Klein EG and Phebus LA. Lanepitant an NK-1 antagonist in migraine prophylaxis. **Cephalalgia** 1999; 19: 377.

Goldstein DJ, Wang O, Saper JR, Stoltz R, Silberstein SD and Mathew NT. Ineffectiveness of neurokinin-1 antagonist in acute migraine: a crossover study. **Cephalalgia** 1997; 17: 785-90.

Gordon ML, Brown SL, Lipton RB. Serotonergic parallels in migraine, depression, and anxiety. Nappi G, Bono G, Sandrini G. Martignoni E, Micieli G, editors. Headache and Depression: **Serotonin Pathways as a Common Clue.** New York: Raven Press, 1991: 21-40.

Gordon ML, Lipton RB, Brown S-L, Nakrascire C, Russell M, Pollack SZ, Korn ML, Merriam A, Solomon S, Van Praag HM. Headache and cortisol responses to m-chlorophen ylpiperazine are highly correlated. **Cephalalgia** 1993; 13: 400-5.

Grailhe R et al. The 5-HT_5 receptors: characterization of the human 5-HT_{5A} receptor, absense of the human 5-HT_{5A} receptor, absense of the human 5-HT_{5B} receptor; knockoul of the mouse 8-HT_{5A} receptor (abstract). **Am.Soc.Neurosci.** 1995; 728 (1): 53-60.

Greene B, Pacitti AJ, Souba WW. Charcterization of L-arginine transport by pulmonary artery endothelial cells. **Am J Physiol** 1993a; 264: L351-6.

Greene-Tallaksen SJ, Young AB, Penney JB and Beitz AJ. Excitatory amino acid binding sites in the trigeminal principal sensory and spinal trigeminal nuclei of the rat. **Neurosci. Lett**. 1993b; 141: 79-83.

Gruetter CA, Kadowitz PJ, Ignarro LJ. Methylene blue inhibits coronary arterial relaxation and guanylate cyclase activation by nitroglycerin, sodium nitrate and amyl nitrate. **Can J Physiol Pharmacol** 1981; 59: 150-6.

Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. **Nature** 1986; 320: 454-6.

Gupta P, Brown D, Butler P, Ellis P, Grayson KL, Land GC, et al. The in vivo pharmacological profile of a 5-HT1 receptor agonist CP122 288 a selective inhibitor of neurogenic inflammation. **Br. J. Pharmacol**. 1995; 116: 2385-90.

Haby K, Lisovoski F, Aunis D, Zwiller. Stimulation of the cyclic GMP pathway by NO induces expression. **J Neurochem** 1994; 62: 496-501.

Haley JE, Dickenson AH, Schachter M. Electrophysiological evidence for a role of nitric oxide in prolonged chemical nociception in rat **Neuropharmacology** 1992; 31: 251-8.

Haley JE, Sullivan AF, Dickenson AH. Evidence for spinal N-methyl-D-aspartate receptor involvement in prolonged chemical nociception in rat. **Brain Res** 1990; 518: 218-26.

Haring JH, Henderson TA, Jacquin MF. Principalis or parabrachial-projecting spinal trigeminal neurons do not stain for GABA or GAD. **Somat Motor Res** 1990; 7: 391-7.

Harrison DG, Bates JN. The nitrovasodilators: New ideas about old drugs. **Circulation** 1993; 87: 1461-7.

Hartig PR, Hoyer D, Humphrey PP, Martin GR. Aligment of receptor nomenclature with the human genome: Classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. **Trends Pharmacol Sci** 1996;17 (3): 103-5.

Hartig PR, Hoffman BJ, Kaufman MJ, Hirato F. The 5-HT_{1C} receptor. **Ann NY Acad Sci** 1996; 600:149-67.

Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. **Cephalalgia** 1988; 8 Suppl 7: 1-96.

Hecker M, Sessa WC, Harris HJ, Anggard E, Vane JR. The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived

relaxing factor: cultured endothelial cells recycle L-citrulline to L-arginine. **Proc Natl Acad Sci USA** 1990; 87: 8612-6.

Hill RG and Salt TE. An iontophoretic study of the responses of rat caudal trigeminal nucleus neurones to non-noxious mechanical sensory stimuli. **J. Physiol**. 1982; 327: 65-78.

Hope BT, Michael GJ, Knigge KM, Vincent SR. Neuronal NADPH diaphorase is a nitric oxide synthase. **Proc Natl Acad Sci** 1991; 88: 2811-4.

Hopf HG, Johnson EA, Gutmann L. Protective effect of serotonin on migraine attacteds. **Neurology** 1992; 42 (7): 1419.

Hoskin KL, Bulmer DC, Goadsby PJ. Fos expression in the trigeminocervical complex of the cat after stimulation of the superior sagittal sinus is reduced by L-NAME**. Neurosci Lett** 1999;266:173-6

Hoskin KL, Kaube H and Goadsby PJ. Central activation of the trigeminovascular pathway in the cat is inhibited by dihydroergotamine a c-Fos and electrophysiology study. **Brain** 1996a; 119: 249-56.

Hoskin KL, Kaube H and Goadsby PJ. Sumatriptan can inhibit trigeminal afterents by an exclusively neural mechanism. **Brain** 1996b; 119: 1419-28.

Hoskin KL, Zagami A and Goadsby PJ. Stimulation of the middle meningcal artery leads to bilateral Fos expression in the trigeminocervical nucleus a comparative study of monkey and cat. **Journal of Anatomy** 1999; 194: 579-588.

Hou M, Kanje M, Longmore J, Tajti J, Uddman R, Edvinsson L. 5-HT(1B) and 5-HT(1D) receptors in the human trigeminal ganglion: co-localization with calcitonin gene-related peptide, substance P and nitric oxide synthase**. Brain Res** 2001; 909(1-2): 112-20.

Hoyer D et al. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). **Pharmacol. Rev** 1993; 46: 157.

Hughes SR, Brain SD. Nitric oxide-dependent release of vasodilator quantities of calcitonin gene-related peptide from capsaicin-sensitive nerves in rabbit skin. **Br J Pharmacol** 1992; 111: 425-30.

Hughes SR, Williams TJ, Brain SD. Evidence that endogenous nitric oxide modulates oedema formation induced by substance P. **Eur J Pharmacol** 1990; 191: 481-4.

Humphrey PPA, Hartig PR, Hoyer DE. A proposed new nomenclture for 5HT receptors. **Trands Pharmacol Sci** 1993; 14: 233-6.

Humphrey PPA. Feniuk W, Marriott AS, Tanner RJN, Jackson MR and Tucker ML. Preclinical studies on the anti-migraine drug, sumatriptan. **Eur. Neurol**. 1991; 31: 282-90.

Humphrey PPA. Feniuk W, Perren MJ, Beresford IJM, Skingle M and Whalley ET. Serotonin and migraine. **Ann. New York Acad. Sci.** 1990; 600: 587-598.

Ialenti A, Ianaro A, Moncada S, Di Rosa M. Modulation of acute inflammation by endogenous nitric oxide. **Eur J Pharmacol** 1992; 211: 177-82.

Ichikawa H, Jacobowitz DM, Suigmoto T. Calretinin-immunoreactive neurons in the trigeminal and dorsal root ganglia of the rat. **Brain Res** 1993; 617: 96-102.

Ignarro LJ, Liptjon H, Edwards JC, Baricos WH, Hyman AL, Kadowitz PJ, Gruetter CA. Mechanisms of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. **J Pharmacol Exp Ther** 1981; 218: 739-49.

Ignarro LJ. Endothelium-derived nitric oxide: pharmacology and relationship to the actions of organic nitrate esters. **Pharmacol Res** 1989; 6: 651-9.

Iversen HK, Holm S, Friberg D. Intracranial hemodynamics during intravenous nitroglycerin infusion. **Cephalalgia** 1989a; 9 Suppl 10: 84-5.

Iversen HK, Nielsen TH, Garre K, Tfelt-Hansen P, Olesen J. Dose-dependent headache response and dilatation of extremity and extracranial arteries after three doses of 5-isosorbi-mononitrate. **Eur J Clin Pharmacol** 1992; 42: 31-5.

Iversen HK, Nielsen TH, Olesen J, Tfelt-Hansen P. Arterial responses during migraine headache. **Lancet** 1990; 336: 837-9.

Iversen HK, Olesen J, Tfelt-Hansen P. Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics. **Pain** 1989b; 38: 17-24.

Iversen HK, Olesen J. Nitroglycerin-induced headache is not dependent on histamine release. Support for a direct nociceptive action of nitric oxide. **Cephalalgia** 1994; 14: 437-3.

Iversen HK, Thomsen LL. Nitroglycerin-induced headache-an experimental headache model. In : Olesen J, Moskowitz MA, editors. **Experimental headache models in animal and man.** New York: Raven Press, 1995: 251-8.

Iversen HK. N-acetylcysteine enhances nitroglycerininduced headache and arterial responses. **Clin Pharmacol Ther** 1992; 52: 125-33.

Jasen K, Tuxen C, Pedersen-Bjerregård U, Jasen I, Edvinsson L, Olesen J. Pain and tnderness in human temporal muscle induced by bradykinin and 5-hydroxytryptamine. **Peptides** 1990; 11: 1127-32.

Jones MG, Lever I, Bingham S, Read S, McMahon SB, Parsons A. Nitric oxide potentiates response of trigeminal neurones to dural or facial stimulation in the rat. **Cephalalgia** 2001;21:643-55

Kai-kai MA. Cytochemistry of the trigeminal and dorsal root ganglia and spinal cord of the rat. Comp. **Biochem. Physiol** 1989; A93: 183-93.

Kanner J, Harel S, Granit R. Nitric oxide, an inhibitor of lipid oxidtion by lipoxygenase, cyclooxygenase and hemoglobin. **Lipids** 1992; 27: 46-9.

Kaube H, Hoskin KL and Goadsby PJ. Activation of the trigeminovascular system by mechanical distension of the superior sagittal sinus in the cat. **Cephalalgia** 1992; 12: 133-6.

Kaube H., Keay K, Hoskin KL, Bandler R and Goadsby PJ. Expression of c-fos-like immunoreactivity in the trigeminal nucleus caudalis and high cervical cord following stimulation of the sagittal sinus in the cat. **Brain Res.** 1993a; 629: 95-102.

Kaube H, Hoskin KL and Goadsby PJ. Sumatriptan inhibits central trigeminal neurons only after blood-brain barrier disruption. **Br. J. Pharmacol.** 1993b.; 109: 788-92.

Kelm M, Feelisch, Grube R, Motz W, Strauer BE. Metabolism of endothelium-derived nitric oxide in human blood. In: Moncada S, Marletta MA, Hibbs JB, Higgs EA, editors. **The biology of nitric oxide, vol. 1**. London: Portland Press, 1992: 319-22.

Kennett G. Serotonin receptor and their function. Available from: **http://www.tocris.com/litrequest.htm (2000).**

Kiechle FL, Malinski T. Nitric oxide. Biochemistry, pathophysiology and detection. **Am J Clin Pathol** 1993; 100: 567-75.

Kimball RW, Friedman AP, Vallejo E. Effect of serotonin in migraine patients. **Neurology** 1960; 10: 107-11.

Kitto KF, Haley JE, Wilcox GL. Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. **Neurosci Lett** 1992; 148: 1-5.

Knight YE, Connor HE, Edvinsson L and Goadsby PJ. Only $5HT_{1B\ 1D}$ agonist doses of 4991W93 inhibit CGRP release in the cat. **Cephalalgia** 1999; 19: 401.

Knight YE, Edvinsson L and Goadsby PJ. Blockade of release of CGRP after superior sagittal sinus stimulation in cat a comparison of avitriptan and CP122288. **Cephalalgia** 1997; 17: 248.

Knight YE, Goadsby PJ. Naratriptan inhibits central trigeminal activity by a $5HT_{1B/1D}$ receptor. **Cephalalgia** 1997; 17: 403.

Knowles RG, Merrett M, Salter M, Moncada S. Differential induction of brain, lung and liver nitric oxide synthase by endotoxin in the rat. **Biochem J** 1990; 270: 833-6.

Knowles RG, Moncada S. Nitric oxide synthases in mammals. **Biochem J** 1994; 298: 249-58.

Knyihar-Csillik E, Vecsei L. Effect of a nitric oxide donor on nitroxergic nerve fibers in the rat dura mater. **Neurosci Let**t 1999;260:97-100

Kohen R et al. Cloning, characterization, and chromosomal localization of a human 5-HT_6 serotonin receptor. **J Neurochem** 1996; 66: 47.

Kolhekar R, Meller ST, Gebhart GF. NMDA receptor-mediated biphasic effects on thermal nociceptive spinal transmission (abstract). **Soc Neurosci** 1992; 18: 1024.

Krabbe AE, Olesen J, Headache provocation by continuous intravenous infusion of histamine. Clinical results and receptor mechanisms. **Pain** 1980; 8: 253-9.

Kuo HP, Liu S, Barnes PJ. The effect of endogenous nitric oxide on neurogenic plasma exudation in guinea-pig airways. **Eur J Pharmacol** 1992; 221: 385-8.

Lance JW, Anthony M and Hinterberger H. The control of cranial arteries by humoral mechanisms and its relation to the migraine syndrome. **Headache** 1967; 7: 93-102.

Lance JW. 5-hydroxytryptamine and its role in migraine. **Eur J Neurol** 1991; 31: 279-81.

Lance JW. History of involvement of 5-Ht in primary headaches. In: Olesen J, Saxena PR, editors. **5-hydroxytryptamine mechanisms in primary headaches.** New York: Raven Press, 1992; 19-28.

Lassen LH, Thomsen LL, Olesen J. Histamine induces migraine via the H1-receptor. Supprot for the NO-hypothesis of migraine. **Neuroreport** 1995; 6: 1475-9.

Lee WK, Limmroth V, Ayata C, Cutrer FM., Waeber C, Yu X.-Y and Moskowitz MA. Peripheral GABA-A receptor mediated effects of sodium valproate on dural plasma protein extravasation to substance P and trigeminal stimulation. **Br. J. Pharmacol**.1995; 116:1661-7.

Leff P, Martin Gr, Morse JM. Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by use of tryptamine analogues. **Br J Pharmacol** 1987; 91: 321-31.

Leonard BE. Sub-types of serotonin receptors: biochemical changes and pharmacologjical consequences. **Int Clin Psychopharmacol** 1992; 7: 13-21.

Leong SK, Liu HP, Yeo JF. Nitric oxide synthase and glutamate receptor immunoreactivity in the rat spinal trigeminal neurons expressing Fos protein after formalin injection. **Brain Res** 2000; 855:107-15.

Lepoivre M, Fieschi F, Coves J, Thelander L, Fontecave M. Inactivation of ribonucleotide reductase by nitric oxide. **Biochem Biophys Res Comm** 1991; 179: 442-8.

Limmroth V, Lee WS, Cutrer FM, Waeber C and Moskowitz MA. GABA-receptor,-mediated effects of progesterone its ring-A-reduced metabolites and synthetic neuroactive steroids on neurogenic oedema in the rat meninges. **Br J Pharmacol** 1996; 117: 99-104.

Lippe IT, Stabentheiner A, Holzer P. Participation of nitric oxide in the mustard oil-induced neurogenic inflammation of the rat paw skin. **Eur J Pharmacol** 1993; 232: 113-20.

Lipton RB, Stewart WF. Prevalence and impact of migraine. **Neurol Clin** 1997; 15 (1): 1-13.

Liu L, Pugh W, Ma H, Simon SA. Identification of acetylcholine receptors in adult rat trigeminal ganglion neurons. **Brain Res** 1993; 617: 37-42.

Longmore J et al. Differential distribution of $5HT1_D$ and $5HT_{1B}$ immunoreactivity within the human trigeminocerebrovascular system: implications for the discovery of new antimigraine drugs. **Cephalalgia** 1997; 17: 833-42.

Lucas JJ, Mellstrom B, Colado MI, Naranjo JR. Molecular mechanism of pain: serotonin 1A receptor agonist triggers transactivation by c-fos of the predynorphin gene in spinal cord neurons. **Neuron** 1993; 10: 599-611.

Magnusson KR, Larson AA, Madl JE, Altschuler RA, Beitz AJ. Co-localization of fixative-modified glutamate and glutaminase in neurons of the spinal trigeminal nucleus of the rat: An immunohistochemical and immunoradiochemical analysis. **J Comp Neurol** 1986; 247: 477-90.

Mamounas LA, Wilson MA, Axt KJ, Molliver ME. Morphological aspects of serotonergic innervation. In: Bradley PB, Handley SL, Cooper SJ, Key BJ, Barnes NM, Coote JH, editors. **Serotonin, CNS receptors and brain function.** New York: Pergamon Press, 1992; 97-118.

Markowitz S, Saito K and Moskowitz MA. Neurogenically mediated leakage of plasma proteins occurs from blood vessels in dura mater but not brain. **J. Neurosci** 1987; 7: 4129-4136.

Marret S, Bonnier C, Raymackers JM, Delpech A, Evrard P and Gressens P. Glycine antagonist and NO degrees, synthase inhibitor protect the developing mouse brain against neonatal excitotoxic lesions. **Pediatric Research** 1999; 45 (3): 337-342.

Martin GR, Browingt C, Giles M. Further characterisation of an atypical 5-HT receptor mediating endothelium-dependent vasorelaxation. **Br J Pharmacol** 1993; 110-37.

May A, Gijsman HJ, Wallnofer A, Jones R, Diemer HC, Ferran MD. Endothelin antagonist Bosentan blocks neurogenic inflammation, but is not effective in aborting migraine attacks. **Pain** 1996; 67: 375-8.

Mayer ML, Westbrook GL. The physiology of excitatory amino acids in the vertebrate central nervous system. **Prog Neurobiol** 1987; 28: 197-276.

Mayer B. Regulation of nitric oxide synthase and soluble guanylyl cyclase. **Cell Biochem Funct** 1994; 12: 167-77.

McCall T, Vallance P. Nitric oxide takes centre-stage with newly defined roles. **Trends Pharmacol Sci** 1990; 13: 1-6.

Meller ST, Dykstra C, Gebhart GF. Production of endogenous nitric oxide and activation of soluble gunylate cyclase are required for N-methyl-D-aspartate-roduced facilitation of the nociceptive tail-flick reflex. **Eur J Pharmacol** 1992a; 214: 93-6.

Meller ST, Pechaman PS, Gebhart GF, Maves TJ. Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. **Neuroscience** 1992b; 50: 7-10.

Meller ST, Gebhart GF. Nitric oxide (NO) and nociceptive processing in the spinal cord. **Pain** 1993; 52: 127-36.

Meller ST, Lewis SJ, Bates JN, Brody MJ, Gebhart GF. Is there a role for an endothelium-derived relaxing factore in nociception?. **Brain Res** 1990; 531: 342-5.

Millan MJ. Kappa opioid receptors and analgesia. **Trands pharmac Sci** 1990; 11: 70-6.

Mills A and Martin GR. Autoradiographic mapping of [^{3}H]sumatriptan binding in cat brain stem and spinal cord. **Eur. J. Pharmacol.** 1995; 280: 175-8.

Mitsikostas DD, Sanchez del Rio M, Wacber C, Moskowitz MA and Cutrer FM. The NMDA receptor antagonist MK 801 reduces capsaicin-induced c-fos expression within rat trigeminal muscleus caudalis. **Pain** 1998; 76: 239-48.

Mitsikostas DD, Sanchez del Rio M, Waeber C, Hang Z, Cutrer FM and Moskowitz MA. Non-NMDA glutamate receptors modulate capsaicin induced c-fos expression within trigeminal nucleus. **Br. J. Pharmacol** 1999; 127: 623-30.

Miyazaki J, Nakanishi S and Jingami H. Expression and characterization of a glycine-binding fragment of the N-methyl-D-aspartate receptor subunit NR1. **Biochemical Journal.** 1999; 340 (Pt3): 687-92.

Mizukawa K, Vincent SR, McGeer PL, McGeer EG. Distribution of reduced-nicotinamide-adenine-dinucleotide-phos-phate diaphorase-positive cells and fibers in the cat central nervous system. **J Comp Neurol** 1989; 279: 281-311.

Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: Physiology, pathophysiology and pharmacology. **Pharmacol Rev** 1991; 43: 109-10.

Moore PK, Oluyomi AO, Babbedge RC, Wallace P, Hart SL. L-N^G-nitro arginine methyl ester exhibits antinociceptive activity in the mouse. **Br J Pharmacol** 1991; 102: 198-202.

Moriguchi M, Manning LR, Manning JM. Nitric oxide can modify amino acid residues in proteins. **Biochem Biophy Res Comm** 1992; 183: 598-604.

Morris R, Southam E, Braid DJ, Garthwaite J. Nitric oxide may act as a messenger between dorsal root ganglion neurones and their satellite cells **Neurosci Lett** 1992; 137: 29-32.

Moskowitz MA. Basic mechanisms in vascular headache. **Neurol Clin** 1990; 8: 801-5.

Moskowitz MA. Interpreting vessel diameter changes in vascular headaches. **Cephalalgia** 1992a; 12: 5-7.

Moskowitz MA. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. **Trends Pharmacol Sci** 1992b; 13: 307-11.

Moskowitz MA. Neurogenic inflammation in the pathophysiology and treatment of migraine. **Neurology** 1993; 43 Suppl 3: S16-20.

Murray CW, Cowan A, Larson AA. Neurokinin and NMDA anatogonists (but not a kainic acid antagonist) are antinociceptive in the mouse formalin model. **Pain** 1991; 44: 179-85.

Nankai M, Klarica M, Fage D and Carter C. The pharmacology of native N-methyl-D-aspartate receptor subtypes. Djifferent receptors control the release of different striatal and spinal transmitters. **Progress in Neuro-Psychopharmacology and Biological psychiatry** 1998; 22 (1): 35-64.

Naranjo JR, Mellstrom B, Achaval M, Sassone-Corsi P. Molecular pathways of pain: fos/jun mediated activation of a noncononical AP-site in the prodynorphingene. **Neuron** 1991; 6: 607-17.

Nicolodi M and Sicuteri F. Relief of migraine attack with N-methyl-d-aspartic acid receptor antagonist ketamine a double blind comparison with placebo-theoretic implications. **Cephalalgia** 1996; 16: 372.

Nilsson T, Longmore J, Shaw D, Jansen-Olesen I and Edvinsson L. Contractile 5-HT_{1B} receptors in human cerebral arteries: pharmacological characterization and localization with immunocytochemistry. **Br. J. Pharmacol.** 1999; 128: 1133-1140.

Norman. B, Panebianco D and Block. GA. A placebo-con-trolled in-clinic study to explore the preliminary safety and efficacy of intravenous L-758,298 (a prodrug of the NK1 receptor antagonist L-754, 030) in the acute treatment of migraine. **Cephalalgia** 1998; 18: 407.

Northfield DWC. Some observations on headache. **Brain** 1938; 61: 133-62.

Nozaki K, Moskowitz MA, Boccalini P. CP93,129, Sumatriptan, dihydroergotamine block c-fos expression with in rat trigeminal nucleus caudalis caused by chemical stimulation of the meninges. **Br J Pharmacol** 1992a; 106: 409-15.

Nozaki K, Boccalini P, Moskowitz MA. Expression of c-fos-like immonoreactivity in brainstem after meningeal irritation by blood in the subarachnoid space. **Neuroscience** 1992b; 49: 669.

Nozaki K, Moskowitz MA, Maynard KI, Koketsu N, Dawson TM, Bredt DS et al. Possible origins and distribution of immunoactive nitric oxide synthetase- containing nerve fibers in cerebral arteries. **J Cereb Blood Flow Metab** 1993; 13: 70-9.

O'Dell TJ, Hawkins RD, Kandel ER, Arancio O. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. **Proc Natl Acad Sci** 1991; 88: 1285-9.

Oka M. Experimental study on the vasodilator innervation of the face (abstract) **Med. J. Osaka Univ** 1950; 2: 109-16.

Olesen J, Iversen HK, Thomsen LL, Nitric oxide supersensitivity. A possible molecular mechanism of migraine pain. **Neuroreport** 1993; 4: 1027-30.

Olesen J, Thomsen LL, Iversen HK. Nitric oxide is a key molecule in migraine and other vascular headaches. **Trends Pharmacol Sci** 1994; 15: 149-53.

Onofrio BM. Radiofrequency percutaneous Gasserian ganglion lesions. Results in 140 patients with trigeminal pain **J. Neurosurg** 1975; 42: 132-43.

Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptors in the mamalian central nervous system. **Prog Neuro**biol 1998; 54: 581-18.

Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. **Nature** 1987; 327: 524-6.

Pascual J, Arco,CD, Romon T, Olmo CD, Pazos A. [^{3}H] Sumatriptan binding sites in human brain regional-dependent labelling of $5HT_{1D}$ and $5HT_{1F}$ receptors. **Eur. J. Pharmacol**. 1996 ; 295: 271-274.

Patel TD et al. Localization of serotonin-4 receptors in the striatonigral pathway in the rat brain. **Neuroscience** 1995; 69: 248-53.

Pedersen-Bjerregård U, Nielse LB, Jensen K, Edvinsson L, Jansen I, Olesen J. Calcitonin gene-related peptide, neurokinin A and substance P: Effects on nociception and neurogenic inflammation in human skin and temporal muscle. **Peptides** 1991; 12: 333-7.

Perren MJ, Feniuk W and Humphrey PPA. Vascular 5-HT_1-like receptors that mediate contraction of the dog isolated saphenous vein and carotid arterial vasoconstriction in anaesthetised dogs are not of the 5-HT1A or 5-HT1D subtype. **Br. J. Pharmacol.** 1991; 102: 191-7.

Phebus LA et al. Characterisation of LY 334370, a potent and selective 5-HT_{1F} receptor agonist, in the neurogenic dural inflammation model of migrain pain (abstract). **Am.Soc.Neurosci** 1996; 22: 35-41.

Pineyro G et al. Regulation of 5-HT release in 5-HT_{1B} knockout mice: experiments in hippocampal, trontal cortex and midbrain raphe slice (abstract). **Am Soc Neurosci** 1995; 21: 539-6.

Proletti-Cecchini P, Afra J and Schoenen J. Intensity dependence of the cortical auditory evoked potentials as a surrogate marker of central nervous system serotonin transmission in man demonstration of a central effect for the $5HT_{1B/1D}$ agonist zolmitriptan (311C90, Zomig). **Cephalalgia** 1997; 17: 849-854.

Raskin NH. Headache. **Compn Ther** 1981; 7 (4): 32-7.

Raskin NH. Serotonin receptors and headache. **New Engl J Med** 1991; 31: 279-81.

Read SJ, Manning P, McNeil CJ, Hunter AJ, Parsons AA. Effects of sumatriptan on nitric oxide and superoxide balance during glyceryl trinitrate infusion in the rat. Implications for antimigraine mechanisms. **Brain Res** 1999;847:1-8

Read SJ, Smith MI, Hunter AJ, Parsons AA. Enhanced nitric oxide release during cortical spreading depression following infusion of glycerly trinitrate in the anaesthetized cat. **Cephalalgia** 1997; 17: 159-65.

Reif DW, Simmons RD. Nitric oxide mediates iron release from ferritin. **Arch Biochem Biophys** 1990; 283: 537-41.

Ren K, Hylden JLK, Williams GM, Ruda MA, Dubner R. The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. **Pain** 1992; 50: 331-44.

Ren K, Williams GM, Hylden JLK, Ruda MA, Dubner R. The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuates carrageenan-induced behavioral hyperalgesia in rats**. Eur J Pharmacol** 1992; 219: 235-43.

Roberts MHT. 5-Hydroxytryptamine in nociception and antinociception. In: Olesen J, Saxena PR,editors. **5-HT Mechanisms of Primary Headaches.** New York: Raven Press, 1992: 69-79.

Rodella L, Rezzani R, Agostini C, Bianchi R. Expression of NADPH-diaphorase and colocalization with Fos in the brain neurons of the rat following visceral noxious stimulation. **Brain Res** 1999;834 (1-2):173-7.

Rozniecki JJ, Kuzminska B, Prusinski A. The possible mechanism of nitroglycerin induced cluster headache attack. A proposal explanation. **Cephalalgia** 1989; 9 Suppl 10: 80-1.

Salt TE and Hill R.G. Differentiation of excitatory amino acid receptors in the rat caudal trigeminal nucleus a microiontophoretic study. **Neuropharmacol.** 1982; 21: 385-90.

Saxcna PR. 5-HT in migraine-an introduction. **J. Neurol** 1991; 238: S36-S37.

Schmetterer L, Wolzt M, Graselli U, Findl O, Strenn K, Simak S, Kastner J, Eichlser HG, Singer EA. Nitric oxide synthase inhibition in the histamine headache model. **Cephalalgia** 1997; 17: 175-82.

Schmidt HW, NO Endogenous soluble guanylyl cyclase-activation factors. **FEBS *Lett*** 1992; 307: 102-7.

Schmidt K, Klatt P, Mayer B. Characterization of endothelial cell amino acid transport systems involved in the actions of nitric oxide synthase inhibitors. **Mol Pharmacol** 1993; 44: 615-21.

Schmuck K, Ullmer C, Engels P, Lübbert H. Cloning and functional characterization of the human 5-HT_{2B} serotonin receptor. **FEBS *Lett*** 1994; 324: 85-90.

Schumacher GA, Wolff HG. Experimental studies on headache. **Arch Neurol Psychiatry** 1941; 45: 199-213.

Schuman EM, Madison DV. A requirement for the intracellular messenger nitric oxide in long-term potentiation. **Science** 1992; 254: 1503-6.

Seeburg PH. The molecular biology of mammalian glutamate receptor channels. **Trends in the Neurosciences** 1993; 16 (9): 359-65.

Shepheard SL, Williamson DJ, Williams J, Hill RG and Hargreaves R. Comparison of the effects of sumatriptan and the NK_1 antagonist CP-99, 994 on plasma extravasation in the dura mater and c-fos mRNA expression in the trigeminal nucleus caudalis of rats. **Neuropharmacol** 1995; 34: 255-61.

Sicuteri F, Testi A and Anselmi B. Biochemical investigations in headache increase in hydroxyindoleacetic acid excretion durign migraine attacks. **International Archives of Allergy** 1961; 19: 55-8.

Sicuteri FE. Del Bene M, Poggioni, Bonazz A. Unmasking latent dysnociception in healthy subjects. **Headache** 1987; 27: 180-5.

Silberstein SD. Serotonin (5-HT) and migraine. **Headache** 1994; 34: 408-17.

Snell RS. The Cranial nerve nuclei and their central connections. In: Snell RS, editor. **Clinical neuroanatomy for medical students**. Brown and company: Little, 1992: 430.

Spira PJ, Mylecharane EJ, Misbach J, Duckworth JW and Lance JW. Internal and external carotid vascular responses to vasoactive agents in the monkey. **Neurology** 1978; 28h: 162-73.

Srikiatkhachorn A, Anuntasethakul T, Maneesri S, Phansuwan-Pujito P, Patumraj S, Kasantikul V. Hyposerotonin-induced nitric oxide supersensitivity in the cerebral microcirculation. **Headache** 2000; 40: 267-75.

Srikiatkhachorn A, Anuntasethakul T, Phansuwan-Pujito P, Patumraj S, Kasantikul V. Effect of Serotonin Depletion on Nitric Oxide Induced Cerebrovascular Nociceptive Response. **Neuroreport** 2001;12: 967-71.

Srikiatkhachorn A, Puangniyom S, Govitrapong P. Plasticity of 5-HT_{2A} serotonin receptor in patients with analgesic-induced transformed migraine. **Headache** 1998; 38: 534-9.

Srikiatkhachorn A, Tarasub N, Govitrapong P. Acetaminophen-induced antinociception via central 5-HT_{2A} receptors. **Neurochem Int** 1999;34:491-8.

Stamler JS, Simon DI, Osborne JA, Mullins ME, Jaraki O, Michel T, et al. S-nitrosylation of proteins with nitric oxide-synthesis and characterization of biologically active compounds. **Proc Natl Acad Sci USA** 1992a; 89: 444-8.

Stamler JS, Single DJ, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. **Science** 1992b; 258: 1898-902.

Stepien A, Chalimoniuk M, Strosznajder J. Serotonin 5-$HT_{1B/1D}$ agonists abolish NMDA-evoked enhancement of nitric oxide synthase and cGMP concentration in brain cortex slices. **Cephalalgia**. 1999;19:859-65.

Steward LJ et al. Evidence that the atypical 5-HT_3 receptor ligand, [^{3}H]-BRL 46470, labels additional 5-HT_3 binding sites compared to [^{3}H]-granisetron. **Br J Pharmacol** 1995; 116 (2): 1781-8.

Storer RJ and Goadsby PJ. Trigeminovascular nociceptive transmission involves NMDA and non-NMDA glutamate receptors. **Neuroscience** 1999; 90: 1371-6.

Storer RJ. Connor HE and Goadsby PJ. Microiontophoretic application of 4991W93 inhibits trigeminocervical neurons. **Cephalalgia** 1999; 19: 314.

Strassman AM, Mineta Y, Vos BP: Distribution of fos-like immnoreactivity in the medullary and upper cervical dorsal horn produced by stimulation of dural blood vessels in the rat. **J Neurosci** 1994; 14: 3725.

Strassman AM, Raymond SA and Burstein R. Sensitization of meningeal sensory neurons and the origin of headaches. **Nature** 1996; 384: 560-3.

Stuehr DJ, Cho HJ, Kwon NS, Weise MF, Nathan CF. Purification and characterization of the cytokine-induced macrophage nitric oxide synthase: an FAD-and FMN-con-taining flavoprotein. **Proc Natl Acad Sci USA** 1991; 88: 773-7.

Sumner MJ. Characterization of 5-HT receptor mediating endothelium-dependent relaxation in porcine vena cava. **Br J Pharmacol** 1991; 102: 938-42.

Sweet WM and Wepsic JG. Controlled thermocoagulation of V ganglion and rootlets for differential destruction of pain fibres. Part I:V. **Neurosurg**. 1974; 40: 143-56.

Synder SH. Nitric oxide: first in a new class of neurotransmitters?, **Science** 1992; 257: 494-6.

Tassorelli C, Joseph SA, Buzzi MG and Nappi G. The Effects on the central nerrous system of nitroglycerin-putative mechanisms and mediators. **Prog Neurobio** 1999; 57: 607-24.

Tassorelli C, Joseph SA. NADPH-diaphorase activity and Fos expression in brain nuclei following nitroglycerin administration. **Brain Res** 1995; 695: 37-44.

Tassorelli C, Joseph SA. Systemic nitroglycerin induces Fos immunoreactivity in brainstem and forebrain structures of the rat. **Brain Res** 1995; 682(1-2): 167-81.

Tfelt-hansen P. Efficacy and adverse events of subcutaneous. Oral, and intranasal sumatriptan used for migraine treatment: a systematic review based on number needed to treat. **Cephalalgia** 1998; 18: 532-8.

The Subcutaneous Sumatriptan International Study Ghroup., Ferrari MD. Treatment of migraine attacks with sumatriptan. **N. Eng. J. Med**. 1991; 325: 316-21.

Thompson SWN, Woolf CJ. Primary afferent-evoked prolonged potentials in the spinal cord and their central summation: role of the NMDA receptor. In: M.R. Bond, J.E. Charlton and C.J. Woolf, editors**. Proc. VI th World Congress on Pain** Elsevier; Ansterdam, 1991: 291-8.

Thomsen LL, Iversen HK, Brinck TA, Olesen J. Arterial supersensitivity to nitric oxide (nitroglycerin) in migraine sufferers. **Cephalalgia** 1993; 13: 395-9.

Thomsen LL, Iversen HK, Olesen J. Cerebral blood flow velocities are reduced during attacks of unilateral migraine without aura. **Cephalalgia** 1995; 158: 109-16.

Thomsen LL, Kruuse C, Iversen HK, Olesen J. A nitric oxide donor (nitroglycerin) triggers genuine migraine attacks. **Eur J Neurol** 1994; 1: 73-80.

Thomsen LL, Olesen J. A pivotal role of nitric oxide in migraine pain. **Ann NY Acad Sci** 1997; 835: 363-72.

Thomsen LL, Olesen J. Nitric Oxide Theory of migraine. **Clinical Neuroscience** 1998; 5: 28-33.

Thomsen LL. Investigations into the role of nitric oxide and the large intracranial arteries in migrain headache. **Cephalalgia** 1997; 17: 873-95.

To ZP, Bonhaus DW, Eglen RM, Jakeman LB. Characterization and distribution of putative 5-HT receptors in guinea pig brain. **Br J Pharmacol** 1995 (1); 115: 107-16.

Toda N. Mechanism underlying responses to histamine of isolated monkey and human cerebral arteries. **Am J Physiol** 1990; 258: H311-7.

Traub RJ, Solodkin A, Gebhart GF. A bilateral increase in NADPM-diaphorase activity in the rat lumbar spinal cord following unilateral hindpay inflammation (abstract). **Soc Neurosci** 1992;18: 135.

Tricklebank MD. The antipsychotic potential of subtype-selective 5-HT receptor ligands based on interactions with mesolimbic dopamine systems. **Behav. Brain Re**s 1996; 73(1-2): 15-7.

Tseng LF, Wang HQ and Xu JY. Inhibition of spinal nitric oxide by N (omega)-nitro-L-arginine blocks the release of Met-enkephalin and antirociception induced by spinally administered beta-endorphin in the rat. **Neuroscience** 1997; 78 :461-7.

Uddman R, Edvinsson L, Ekman R. Innervation of the feline cerebral vasculature by nerve fibers containing calcitonin gene-related peptide: Trigeminal origin and co-existence with substance P. **Neurosci Lett** 1985; 62: 131.

Ullmer D et al. 5-HT_{2B} receptor-mediated calcium release from ryanodine-sensitive intracellular stores in human pulmonary artery endothelial cells. **Br J Pharmacol** 1996; 117 (6): 1081-8.

Valtschanoff JG, Weinberg RJ, Rustioni A. NADPH diaphorase in the spinal cord of rats. **J Comp Neurol** 1992; 320: 1-4.

Venturini CM, Palmer RMJ, Moncada S. Vascular smooth muscle contains a depletable store of a vasodilator which is light-activated and restored by donors of nitric oxide. **J Pharmacol Exp Ther** 1993; 266: 497-500.

Villalon CM, Bom AH, Boer MOD, Heiligers JP and Saxena PR. Effects of S9977 and dihydroergotamine in an animal experimental model for migraine. **Pharmacol Res** 1992; 25: 125-37.

Vincent SR, Kimura H. Histochemical mapping of nitric oxide synthase in the rat brain. **Neuroscience** 1992; 46: 755-84.

Waite PME, Tracey David. Trigeminal sensory system. In : Paxinos G, editor. **The rat nervous system** New York: Academic Press, 1995: 705-24.

Wallace MN, Bisland SK. NADPH-diaphorase activity in activated astrocytes represents inducible nitric oxide synthase. **Neuroscience** 1994; 59: 905-19.

Wang H, Nie H, Zhang RX, Qiao JT. Peripheral nitric oxide contributes to both formalin- and NMDA-induced activation of nociceptors: An immunocytochemical study in rats. **J Neurosci Res** 1999; 57: 824-9.

Wang W, Timsit-Berthier M and Schoenen J. Intensity dependence of the auditory cortical evoked potentials is pronounced in migraine: an indication of cortical potentiation and low serotonergic transmission? **Neurology** 1996; 46: 1404-9.

Wei EP, Moskowitz MA, Baccalini P, Kontos HA. Calcitinon gene related peptide mediates nitroglycerin and sodium nitroprusside induced vasodilation in feline cerebral arterioles. **Circ Res** 1992; 70 :1313-9.

Weiller C, May A, Limmroth V, Juptner M, Kaube H, Schayck RV, et al. Brain stem activation in spontaneous human migraine attacks. **Nature** 1995; Medicine 1: 658-660.

Wenmalm A, Peterson A. Analysis of nitrite as a marker for endothelium-derived relaxing factor in biological fluids using electron paramagnetic resonance spectrometry. **J Cardiovasc Pharmacol** 1991; 17: S34-40.

Wiesenfeld-Hallin Z, Hökfell T, Lundberg JM, Firssmann WG, Revneche M, Tschopp FA, Fischer JA. Immunoreactive calcitonin gene-related peptid and substance P coexist in sensory nevrons to the spinal cord and interact in spinal behavioral responses of the rat. **Neurosci Lett** 1984; 52, 199-204.

Williamson DJ, Hargreaves RJ, Hill RG and Shepherad SL. Intravital microscope studies on the effects of neurokinin agonists and calcitonin gene-related peptide on dural blood vessel diameter in the anaesthetized rat. **Cephalalgia** 1997a; 17: 518-524.

Williamson DJ, Hargreaves RJ, Hill RG and Shepheard SL. Sumatriptan inhibits neurogenic vasodilatation of dural blood vessels in the anaesthetized rat-intravital microscope studies. **Cephalalgia** 1997b; 17: 525-531.

Williamson DJ, Shepheard SL, Hill RG and Hargreaves RJ. The novel anti-migraine agent rizatriptan inhibits neurogenic dural vasodilatation and extravasation. **Eur J Pharmacol** 1997c ; 328: 61-64.

Willis WD, Westlund KN, Carlton SM. Pain. In: Paxinos G, editor. **The rat nervous system.** New York: Academic Press, 1995: 705-24.

Wolf G, Würdig S, Schünzel G. Nitric oxide synthase in rat brain is predominantly located at neuronal endoplasmic reticulum: an electron microscopic demonstration of NADPH-diaphorase activity. **Neurosci Lett** 1992; 147: 63-6.

Woolf CJ, Thompson SWN. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation: implications for the treatment of post-injury pain hypersensitivity states. **Pain** 1991; 44: 293-300.

Woolf CJ. Excitability changes in central neurons following peripheral damage: the role of central sensitization in the pathogenesis of pain. In: WD Willis, editor. **Hyperalgesia and Allodynia.** New York: Raven Press,1992: 221-44.

Woolf CJ. Recent advances in the pathophysiology of acute pain. **Br J Anaesth** 1989; 63: 139-46.

Wu J, Fang L, Lin Q, Willis WD. Fos expression is induced by increased nitric oxide release in rat spinal cord dorsal horn. **Neuroscience** 2000; 96 (2): 351-7.

Yoskimura M, North RA. Substantia gelatinosa neurons hyperpolarizad in vitro by enkephalin. **Nature** 1983; 305: 529-30.

Young HM, Furness JB, Shuttleworth CWR, Bredt DS, Snyder SH. Co-localization of nitric oxide synthase immunoreactivity and NADPH diphorase histochemistry in neurons of the guinea-pig intestine. **Histochemistry** 1992; 97: 375-8.

Yu X.-J, Waeber C, Castanon N, Scearce K, Hen R, Macor JE, et al., 5-carboxamido-tryptamine CP-122,288 and dihydroergotamine but not sumatriptan CP-93,129 and serotonin-5-O-carboxymethyl-glycyl-tyrosinamide block dural plasma protein extravasation in knockout mice that lack 5-hydroxytryptamine$_{1B}$ receptors. **Molecular Pharmacology** 1996; 49: 761-5.

Zagami AS, Goadsby PJ and Edvinsson L. Stimulation of the superior sagittal sinus in the cat causes release of vasoactive peptides. **Neuropeptides** 1990; 16: 69-75.

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