ผลของการให้ยาไดไฮโดรเออโกทามีนในระยะเฉียบพลันและระยะยาว
ต่อปรากฏการณ์คอร์ติคัลสเปรดดิงดีเพรสชันและระบบการรับความเจ็บปวดไตรเจมินัล

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

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EFFECT OF ACUTE AND CHRONIC ADMINISTRATION OF DIHYDROERGOTAMINE ON CORTICAL SPREADING DEPRESSION AND TRIGEMINAL NOCICEPTION

Miss Suteera Vibulyaseck

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Medical Science
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สุธีรา วิบูลยเสข : ผลของการให้ยาไดไฮโดรเออโกทามีนในระยะเฉียบพลันและระยะยาวต่อปรากฏการณ์คอร์ติคัลสเปรดดิงดีเพรสชันและระบบการรับความเจ็บปวดไตรเจมินัล. (EFFECT OF ACUTE AND CHRONIC DIHYDROERGOTAMINE ADMINISTRATION ON CORTICAL SPREADING DEPRESSION AND TRIGEMINAL NOCICEPTION) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ศ.นพ.อนันต์ ศรีเกียรติขจร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ.ดร.ศักนัน พงศ์พันธุ์ผู้ภักดี, 60 หน้า.

ไดไฮโดรเออโกทามีน เป็นอนุพันธุ์ของยาในกลุ่มเออร์กอท อัลคาลอยด์ ซึ่งออกฤทธิ์โดยลดการขยายตัวของหลอดเลือด และสามารถลดอาการปวดศีรษะในผู้ป่วยไมเกรนได้ ผลของการได้รับยาชนิดนี้อย่างต่อเนื่อง ยังไม่เคยมีการศึกษามาก่อน การศึกษาครั้งนี้มีวัตถุประสงค์ เพื่อศึกษาผลของการได้รับยาไดไฮโดรเออโกทามีนในระยะเฉียบพลัน และระยะต่อเนื่องในสัตว์ทดลองที่ถูกกระตุ้นให้เกิดปรากฏการณ์คอร์ติคัลสเปรดดิงดีเพรสชันและศึกษาการเปลี่ยนแปลงของระบบการรับความเจ็บปวดไตรเจมินัล หนูแรทสายพันธุ์วิสต้าร์ถูกนำมาใช้ในการศึกษาครั้งนี้ โดยแบ่งออกเป็นกลุ่มที่ได้รับยาไดไฮโดรเออโกทามีนในระยะเฉียบพลันขนาด 100 ไมโครกรัมต่อน้ำหนักตัว 1 กิโลกรัมทางเส้นเลือดดำระหว่างที่เกิดปรากฏการณ์คอร์ติคัลสเปรดดิงดีเพรสชันและกลุ่มที่ได้รับยาในขนาดที่เท่ากันทางช่องท้องเป็นระยะเวลาต่อเนื่อง 0, 7, 14, และ 28 วัน สารละลาย 0.1% ไดเมธิวซัลฟอกไซด์ใน 0.9% ซาไลน์ในขนาดที่เท่ากันกับยาไดไฮโดรเออโกทามีนถูกใช้ในกลุ่มควบคุมของแต่ละกลุ่ม ปรากฏการณ์คอร์ติคัลสเปรดดิงดีเพรสชันถูกเหนี่ยวนำให้เกิดในหนู โดยการวางผลึกโพแทสเซียมคลอไรด์ขนาด 3 มิลลิกรัมบนผิวสมองใหญ่ส่วนเทมโพรัลด้านขวา การเปลี่ยนแปลงของคลื่นไฟฟ้าบนผิวสมองใหญ่ถูกแปรสัญญาณออกมาโดยใช้แท่งแก้วขนาดเล็กวางที่สมองส่วนหน้าในด้านเดียวกันกับที่กระตุ้น การศึกษาในระบบการรับความเจ็บปวดไตรเจมินัลสามารถดูการเปลี่ยนแปลงการแสดงออกของโปรตีนฟอส ซึ่งเป็นตัวบ่งชี้การทำงานของเซลล์ประสาท ที่กลุ่มเซลล์ประสาทไตรเจมินัลนิวเคลียสคอร์ดัลลิสในก้านสมองส่วนปลาย โดยใช้เทคนิคเวสค์เทิร์น บลอท ผลการศึกษาพบว่า ผลึกโพแทสเซียมคลอไรด์ขนาด 3 มิลลิกรัม สามารถกระตุ้นให้เซลล์ในสมองใหญ่เกิดดีโพลาร์ไรซ์เซชัน ซึ่งเป็นลักษณะของปรากฏการณ์คอร์ติคัลสเปรดดิงดีเพรสชัน โดยในกลุ่มของหนูที่ได้รับยาไดไฮโดรเออโกทามีนในระยะเฉียบพลัน พบว่า ไม่มีการเปลี่ยนแปลงในตัวแปรต่างๆ ของคลื่นดีโพลาร์ไรเซชัน ในขณะที่การแสดงออกของโปรตีนฟอสในก้านสมองส่วนปลายนั้นลดลง เมื่อเทียบกับกลุ่มควบคุม ในส่วนของหนูกลุ่มที่ได้รับยาในระยะเวลาต่อเนื่องทุกวัน พบว่า พื้นที่ใต้กราฟของคลื่นดีโพลาร์ไรเซชันมีการเปลี่ยนแปลงโดยมีขนาดที่เพิ่มขึ้น ในหนูที่ได้รับยาเป็นเวลา 14 และ 28 วัน เมื่อดูการแสดงออกของโปรตีนฟอสในก้านสมอง พบว่า มีผลที่ไปในทิศทางเดียวกันกับการวัดคลื่นไฟฟ้าบนผิวสมอง โดยกลุ่มที่ได้รับยาต่อเนื่อง 14 และ 28 วันมีการแสดงออกของโปรตีนฟอสเพิ่มขึ้น จากการค้นพบเหล่านี้ แสดงให้เห็นว่า การได้รับยาไดไฮโดรโกทามีนเป็นระยะเวลาต่อเนื่อง สามารถเพิ่มการทำงานของเซลล์ประสาท บริเวณผิวสมองใหญ่และในระบบการรับความเจ็บปวดไตรเจมินัลในบริเวณก้านสมอง โดยสามารถถูกกระตุ้นได้ง่ายขึ้น ผลจากการศึกษาครั้งนี้ สามารถช่วยให้เข้าใจผลทางเภสัชวิทยาและบทบาทของยาไดไฮโดรเออโกทามีน ที่สามารถกระตุ้นให้เกิดอาการปวดศีรษะได้มากขึ้นเมื่อได้รับยาเป็นเวลานาน

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SUTEERA VIBULYASECK : EFFECT OF ACUTE AND CHRONIC DIHYDROERGOTAMINE ADMINISTRATION ON CORTICAL SPREADING DEPRESSION AND TRIGEMINAL NOCICEPTION. ADVISOR : PROF. ANAN SRIKIATKHACHORN, M.D., CO-ADVISOR: ASST. PROF. SAKNAN BONGSEBHANDHU-PHUBHAKDI, Ph.D., 60 pp.

Dihydroergotamine (DHE) is a derivative of ergot alkaloids. Its efficacy is used for vasoconstriction of cranial vessels and can reduce pain in migraine headache. The overuse of DHE is still uncertain. The objective of this study was to investigate the effect of acute and chronic DHE administration in animal induced cortical spreading depression (CSD) and trigeminal nociception. The experiment was divided into 2 groups; the acute-treated group, Wistar rats were received 100 µg/kg DHE, i.v., after the 3rd depolarization waves of CSD was generated and the chronic-treated group, rats were received a once daily 100 µg/kg DHE, i.p., for 0, 7, 14, and 28 days. In the chronic-treated group, the last administration was performed at 30 minutes before CSD induction. The 0.1% dimethyl sulfoxide (DMSO) in 0.9% normal saline was given in the control rats of each group as a vehicle. CSD was induced using application of 3 mg solid potassium chloride and electrocorticogram was recorded within 1 hour and 2 hours for chronic-treated group and acute-treated group, respectively. Trigeminal nociception was studied in brain stem containing trigeminal nucleus caudalis (TNC). The expression of c-Fos protein was examined using western blot analysis. The results showed that acute treatment with DHE significantly decreased the quantity of c-Fos in TNC without the change in CSD patterns. On the contrary, chronic treatment with DHE presented the significant increase in area under the curve (AUC) of CSD waveforms and c-Fos in TNC for 14 and 28 days. These finding indicate that the chronic treatment with DHE can increase the cortical activity and c-Fos expression in trigeminal nociceptive system. This study could support the understanding of DHE-induced medication overuse headache and the pharmacological effect of this drug's efficacy.

Field of Study : Medical Science Student's Signature ....

Academic Year : 2012 Advisor's Signature ....

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LIST OF ABBREVIATIONS

5HT	5-hydroxytryptamine receptor
AgCl	silver chloride
ANOVA	analysis of variance
ATP	adenosine triphosphate
AUC	area under the curve
BCA	bicinchoninic acid
CALCRL	calcitonin receptor-like receptor
CDH	chronic daily headache
CGRP	calcitonin gene-related peptide
CSD	cortical spreading depression
DC	direct current
DHE	dihydroergotamine
DMSO	dimethyl sulfoxide
i.p.	intraperitoneal
i.v.	intravenous
KCl	potassium chloride
kDa	kilodalton
kg	kilogram
µg	microgram
MOH	medication overuse headache
NKA	neurokinin A
NMDA	N-Methyl-D-aspartate
nNOS	neuronal nitric oxide synthase
mV	millivolt
n	number of rat

NSAIDs	non-steroidal anti-inflamatory drugs
PVDF	polyvinylidene fluoride
RAMP1	a receptor activity-modifying protein
RIPA	radioimmunoprecipitation buffer
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SP	substance P
SSS	superior sagittal sinus
TBST	tris-buffered saline with tween
TEMED	tetramethylethylenediamine
TG	trigeminal ganglion
TNC	trigeminal nucleus caudalis

CHAPTER I

INTRODUCTION

Medication overuse headache (MOH) occurs in patients with migraine that transform from episodic to chronic daily headache. MOH is defined as frequent and severe headache that causes from excessive medication usage. MOH patients also experience an increase in psychological disorders (Kaji and Hirata, 2009). Recently, MOH became a major health problem around the world, because MOH patients have been increased continuously. Clinical studies indicated that acute migraine medication including analgesics, triptans, and ergotamines have high risk for MOH development (Krymchantowski, 2003).

Dihydroergotamine (DHE), a derivative of ergotamine, has been used for specific acute migraine treatment for a long time, while it has poor oral availability (less than 1%) (Saper and Silberstein, 2006). DHE possesses a high potency in vasoconstriction effect. DHE binds to receptors including serotonin, noradrenaline, and dopamine (Dahlof and Maassen Van Den Brink, 2012).

Consequently, it is suggested that DHE should be administered parenterally to reach maximal efficacy. Intravenous, intramuscular, and subcutaneous DHE administration are effective in aborting migraine attack, while these administrations are considered to be invasive and unsuitable for patients' self-administration. Recently, an orally inhaled DHE has also been introduced with higher clinical efficacy over placebo studied in a clinical trial (Aurora et al., 2011). Unlike intranasal DHE, which has an inconsistent efficacy, orally inhaled DHE has provided consistent and prolonged pain relief. Because of its efficacy and convenience allows the patients to self-medicate, the possibility of DHE-induced MOH particularly increases in those who inhaled DHE overuse. However, a temporal correlation between DHE exposure

and MOH remains uncertain. We hypothesized that acute DHE suppresses cortical excitability, in contrast to chronic DHE that increases cortical excitability.

In this study, we investigated the effect of acute and chronic DHE administration that leads MOH. Cortical spreading depression (CSD), an underlying phenomenon of migraine aura was used as the experimental model to evaluate cortical excitability. In addition, c-Fos in trigeminal nucleus caudalis (TNC) was used to investigate the level of trigeminal nociception.

CHAPTER II

LITERATURE REVIEWS

Migraine

Migraine is the most common neurological headache. The dominant feature is unilateral headache including throbbing pain. The associated symptoms of migraine include nausea, vomit, photophobia, phonophobia, motor weakness, and sensory disturbance. Migraine can occur with other psychological disorders such as anxiety and depression (Ligthart et al., 2013). Migraine is classified in 7 subclasses; migraine without aura, migraine with aura, childhood periodic syndromes, retinal migraine, complications of migraine, migraine-triggered seizure and probable migraine. Before migraine attacks, there are premonitory symptoms called prodromal phase that the emotional, speech, and sensory will be disturbed. Some patients have partial loss in visual field in aura phase. The aura phase occurs in migraine with aura which is characterized by the aura symptoms. The cause of migraine is not fully known. The pathophysiology of migraine may involve with trigeminal system within the brain. The sensitization of trigeminal system is believed to be the cause of migraine with aura. In early stage, migraine is believed to be triggered by peripheral sensitization. The intracranial structures, activation of neurons, vascular, sensory nerves and some neurotransmitters are mutual related. Accordingly, glutamate sensitivity of NMDA receptors and nNOS activity, which have the important roles in activation of central nervous system, are increased that results to cutaneous allodynia (Burstein et al., 2000). There are many reports has been suggested that serotonin plays the important role in migraine headache by controlling vasodilation and vasoconstriction (Hamel, 2007; Supornsilpchai et al., 2006; Johnson et al., 1998). During migraine attack, neurochemical imbalances involving low serotonin level can facilitate the activation of trigeminal system.

The trigeminal system

The pathophysiology of migraine is used to be explained by trigeminal activation. The animal model has been developed to study the mechanism that underlines the activation of trigeminal system. The trigeminal system consists of trigeminal nerves which innervates the cranial vascular and be activated by sensory input. When the peripheral trigeminal nerve receives the pain perception, it sends the information to trigeminal ganglion which contains the first order neuron. The caudal part (nucleus caudalis) of the spinal trigeminal nucleus is considered to be the site of the second order neurons of the nociceptive pathways of migraine headache. Then, nociceptive signal is sent from TNC to the third order neuron, which locates in the posteromedial nucleus of thalamus, and projected to the primary sensory cortex.

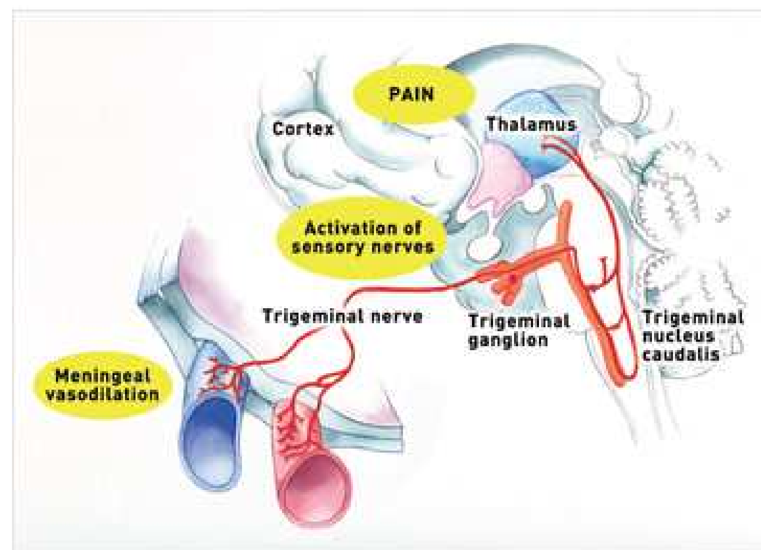

Figure 2.1 The trigeminal system and its activation.

Indeed, the trigeminal system is believed to be related with migraine with aura. When an aura occurs on the cortex of the brain in aura phase of migraine with aura, the excitability of trigeminal neurons is elevated. After that, the peripheral vessels are dilated that leads to release of inflammatory mediators. Most of inflammatory mediators are peptides, such as calcitonin gene-related peptide (CGRP), substance P (SP) and neurokinin A (NKA). These peptides have important

roles in trigeminal system (Tepper et al., 2001). Those releases results in vasodilation of blood vessels, disruption of blood brain barrier, neurogenic inflammation, and trigeminal activation. Animal models of trigeminal activation are used for searching the mechanism and studying the effect of migraine drugs. The activation of trigeminal system by electrical, chemical, and mechanical stimuli in the cranial structure leads to activate of Fos protein expression, a marker for neuronal activity in TNC and cortex (Mitsikostas and Sanchez del Rio, 2001). In addition, alteration of trigeminal system is thought to relate with cortical spreading depression (CSD) that is a phenomenon resulted in the alteration in electrical activity on the cortex.

Cortical spreading depression

In 1944, Leao discovered spreading depression phenomenon by inserting the AgCls electrode in rabbit cerebral cortex that was the continuous wave of CSD characterized by slow wave propagation (Leao, 1944.). However, the cause of CSD is not clearly known. Genetic and environmental factors can activate and develop CSD by changing threshold and susceptibility of the cortex (Eikermann and Ayata, 2010). CSD is believed in underlying of pathogenesis in aura phase of migraine with aura and lead up to delay the activation of trigeminal pathway (Zhang et al., 2010). CSD is the depolarizing wave of neurons and glial cells in the cortex followed by the depression of electrical activity of these cells. CSD starts at primary visual cortex and move forward to frontal pole at a rate of 3-5 mm/min. During aura phase of migraine with aura, CSD can decrease the resistance of neuronal membrane leading to disturbance of ions homeostasis in the brain. Experimental studies showed that the stimulation of the cortex led to the cortical hyperexcitability (Supornsilpchai et al., 2010). There are ionic distribution changes between intracellular and extracellular compartment such as the efflux of K^+ and H^+ to extracellular compartment whereas Na^+, Ca^{2+}, and Cl^- increase in intracellular compartment. The changes of ionic distribution produce the depolarization shift which can observe in DC (direct current)

shift recording. Electrical stimulation, chemical stimulation such as glutamate or K^+ application, mechanical stimulation by light touch or pinprick and brain injury such as trauma, hemorrhage, stroke, and ischemia can be also evoked CSD. In the experimental, CSD susceptibility is used for investigating the mechanism of migraine with aura in rabbit, rat, and cat.

Several studies reported the relationship between CSD and trigeminal system (Pietrobon and Moskowitz, 2013). CSD activates the meningeal nociceptor. That results in neuronal activation and peripheral sensitization of the trigeminal system (Nilsson et al., 2010). CSD stimulates the expression of c-Fos protein which is a marker protein of neuronal activity in trigeminal nucleus caudalis. In addition, central sensitization is enhanced by plasma protein extravasation mediated from dura mater and blood vessels that resulted to hypersensitivity of pain perception. CSD also changes cerebral blood flow through modulating of AMPA and GABA receptors (Holland et al., 2010), but it does not relate to nitric oxide inhibition (Lauritzen, 1994; Zhang et al., 1994).

The expression of c-Fos protein

The c-Fos protein is a proto oncogene which responses as a marker of neuronal activation. It is observed in normal cell type including neurons and their supporting cells. The c-Fos protein can be induced with various stimuli such as electrical stimulation of trigeminal ganglion, chemical stimulation of the meninges, and induction of CSD. CSD induction by electrical, chemical, and mechanical stimuli can activate the nerves surrounding meninges and blood vessels. The activation of neurons and perivascular cells can induce c-Fos expression within brain stem region via trigeminal system. The expression of c-Fos protein is found at ipsilateral ventrolateral (lamina 1-2) of TNC which locates in lower brainstem and upper cervical spinal cord. Therefore, c-Fos is used as a marker of neuronal activity within the processing of nociceptive information (Moskowitz, 1993). The study of the antimigraine drug showed that the c-Fos expression can decrease after treatment in

cats with superior sagittal sinus stimulation (Hoskin et al., 1996; Kaube and Goadsby, 1994).

There are many receptors that involve with c-Fos expression in TNC. Capsaicin is used to study the c-Fos expression in TNC by intracisternal injection (Cutrer, 1995), because it activates the TRPV1 receptor in nerve fibers and c-Fos expression in TNC which can decrease after nociceptin/orphanin FQ administration (Bongsebandhu-Phubhakdi et al., 2011). Moreover, CGRP can activate c-Fos protein in the central neurons of TNC via a G protein-coupled receptor called calcitonin receptor-like receptor (CALCRL) and a receptor activity-modifying protein (RAMP1) (Chatchaisak et al., 2013). NMDA receptor activity that resulted from applying inflammatory soup to 5-HT depletion rats also increases c-Fos expression (Maneepark et al., 2009). Recently, c-Fos expression model has been used to indicate the activity of neurons in trigeminal system. However, it is necessary to co-study with other experiments such as electrophysiological studies for investigating the role of receptors or the efficacy of drug on trigeminal nociception pathway (Mitsikostas and Sanchez del Rio, 2001).

Migraine medications

Migraine can be stimulated by external factors such as food, stress, alcohol consumption, and physical illness. The objective of migraine medications is for relieving pain during migraine attacks and preventing recurrent headache. There are many drugs used for migraine which are divided into several groups according to mechanisms of the action. The general medications for migraine patients are analgesics, opioids, triptans and ergot alkaloids. The efficacy of drugs and side effects are the need to be considered in each patient. Recently, prophylactic drugs such as beta-blockers, calcium-channel blockers, tricyclic antidepressants, anticonvulsants, non-steroidal anti-inflammatory drugs (NSAIDs), angiotensin blockade agents and serotonergic drugs has developed to prevent migraine attack. These preventive

migraine drugs have different mechanisms that control neurotransmitter affecting migraine attack (Modi and Lowder, 2006). CSD is used for a model to investigate the pathophysiology and the pharmacokinetics of migraine drugs. Drugs treatment which interacting with CSD can modify the brain functions with reducing the hyperexcitability of neurons or inhibiting glutamate release in the synaptic terminal (Casucci et al., 2008). The drug mechanism involes the modulation of ion channel, neurotransmitter, receptors and the site of action in the brain which implicates in CSD. Many studies hypothesized that the antimigraine drugs can elevate the CSD threshold that reduced the frequency of migraine attacks (Tozzi et al., 2012). However, it has been reported that the treatment of paracetamol can reduce c-Fos expression in trigeminal nociception, although it has no change on CSD (Supornsilpchai et al., 2010). Additionally, serotonin has the potential for migraine treatment because it affects on the cranial vessels through their receptors. Triptans and ergot alkaloids have been reported as migraine treatment (Evers et al., 1999).

Dihydroergotamine

In 1938, Wolff assumed the vascular theory that initiate cerebral vasoconstriction and followed by extracranial vasodilation (Tfelt-Hansen and Koehler, 2008). This symptom can treat with intravenous ergotamine ($C_{33}H_{35}N_5O_5$) which decreases the temporal pulsation and headache. Furthermore, the studies in vessels of animal and human show the vasoconstriction effect of ergotamine. Dihydroergotamine (DHE; $C_{33}H_{37}N_5O_5$) is a small molecule that is synthesized from ergotamine. DHE is believed to have more efficacy than ergotamine in the treatment of acute migraine attack. DHE possibly effects on central trigeminal pain pathway. In addition, it also affects on blood pressure (Lynch et al., 2010), deep venous thrombosis (Perhoniemi et al., 1996), hypotension blood circulation in retinal (Moreau and Pichon, 1967) and dural plasma extravasation after stimulation on trigeminal ganglion (Markowitz et al., 1988).

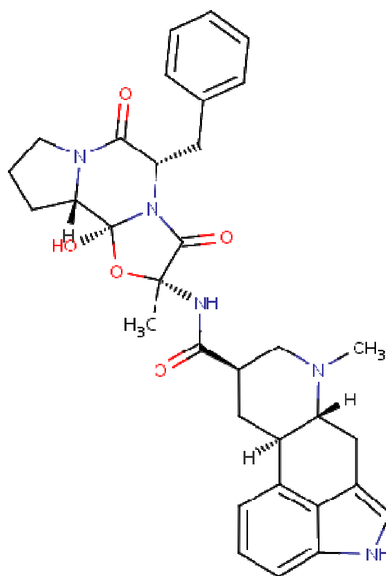

Figure 2.2 The chemical structure of dihydroergotamine.

The bioavailability of DHE depends on the route of administration. The peak plasma levels increases after DHE administration in 1-2 minutes for intravenous, 24 minutes for intramuscular, and 30-60 minutes for intranasal administration. The mean half life is 0.7-1 and 10-13 hours (Silberstein and McCrory, 2003; Dahlof and Maassen Van Den Brink, 2012). The wide range of the mean half life can be the advantage in the recurrent headache treatment. The study using tritium radio-ligand binding with DHE showed that DHE can overspread in many region of the brain, such as, olfactory bulb, frontal cortex, parietal cortex, occipital cortex, cerebellum, mid-brain, and brain stem (Wang et al., 1998).

DHE is accepted for the acute treatment of migraine with aura and cluster headache. Its selectivity is less causing the risk on central nervous system, cardiovascular, and general weakness. The dosage, duration, and plasma concentration are important to be concerned for using. The study of DHE in cat suggested that DHE cannot modulate the propagation of CSD after the burst like single unit cell firing (Kaube and Goadsby, 1994). In the contrast, DHE affects in the trigeminal pathway. That reduces the c-Fos expression in lamina 1 and 2 of TNC after the stimulation of superior sagittal sinus (SSS) (Hoskin et al., 1996). Furthermore,

DHE has the affinity on several receptors. It binds with high affinity to 5-HT receptors, alpha-adrenergic receptors, and dopamine receptors. DHE also has the high binding affinity to alpha2-adrenoreceptor that can be activated and expressed on trigeminal ganglion neuron by changing calcium influx in neuronal cells. DHE can decrease CGRP releasing, block ATP mediated sensitization of trigeminal neurons and decrease P2X3 membrane expression between migraine attacks (Masterson and Durham, 2010).

Table 2.1 Pharmacological activity of DHE, ergotamine, and sumatriptan (Dahlof and Maassen Van Den Brink, 2012; Perrin, 1985).

Pharmacological activity of DHE, ergotamine, and sumatriptan at animal or human monoamininergic receptors, Values for DHE, ergotamine, and sumatriptan are affinity IC_{50} (nM)*			
Receptor	DHE	Ergotamine	Sumatriptan
$5HT_{1A}$	+++	+++	++
$5\text{-}HT_{1B}$	+++	+++	+++
$5\text{-}HT_{1D}$	+++	+++	+++
$5\text{-}HT_{1E}$	+	+	+
$5\text{-}HT_{1F}$	+	+	++
$5\text{-}HT_{2A}$	++	N.D.	0
$5\text{-}HT_{2C}$	+++	N.D.	0
$5\text{-}HT_{3}$	0	0	0
$5\text{-}HT_{4}$	+	+	0
α_{1a}	++	++	0
α_{1b}	++	++	0
α_{2a}	+++	N.D.	0
α_{2b}	+++	N.D.	0
α_{2c}	+++	N.D.	0
β_{1}	0	N.D.	0
β_{2}	0	N.D.	0
β_{3}	+	N.D.	0
D_{2}	+++	+++	0
D_{3}	++	+++	0
D_{4}	++	+++	0

Values for N.D. = not determined.

+++, ++, + and 0 indicate IC50 affinities (nM)

+++ = <5 nM, ++ = >5 to <50 nM, + = >50 to <1500 nM, 0 = >1500 nM

Chronic daily headache and medication overuse headache

Chronic daily headache (CDH) is a symptom characterized by long duration of headache for 15 days or more per months and at least three months. CDH is divided into short and long period of headache. For short period, headache is less than 4 hours of headache such as cluster headache. There are four types of CDH for a long period including chronic migraine, hemicrania continua, chronic tension-type headache (CTTH), and new daily persistent headache (NDPH). CDH is associated with medication overuse headache (MOH) (Ahmed et al., 2012).

Transformed migraine to CDH occurs when medication use increase to the level of overuse. Characteristic symptoms of chronic migraine are more frequent over months to years with the other associated symptoms. The duration of headache which more than 4 hours per day and the medication use for more 15 days per month can lead to MOH. The International classification of headache disorders (ICHD-ll) characterizes the MOH that headache occurs for 15 days or more per month with the overuse of one or more drugs for more than 10 days per months (triptans, ergotamine, opioids, and combination of acute medications with analgesics) and more than 15 days per months for analgesics and NSAIDs.

Recently, there have been many medications for migraine treatment such as analgesics, opioids, NSAIDs, triptans, and ergot alkaloids. The objectives of migraine medications are for reduce the frequency, severity, and disability associated with daily headaches. The excessive use of these medications can lead to MOH. Clinical features of MOH depend on headache frequency, duration of treatment, and the substances class. During MOH, patients cannot use the drugs in the same doses and the symptoms would worsen during medication uses. The phychological symtoms can occur in MOH because some drugs have the composition of mental substances such as cafein, codein, and barbiturate.

MOH can be caused by the drug intake which includes analgesic, opioids, triptans, ergotamine, and combination medications. The overuse of dihydroergotamine was reported several years ago (Tfelt-Hansen and Koehler,

2008), patients had recurrent migraine headache and got worse. Adverse effects of dihydroergotamine in excessive use causes the symptoms of nausea, acroparesthesia, ischemia, habituation, overuse headache, and ergotism. In 1997, it was reported that the association symptoms of migraine were induced when patient has excessive dosage or chronic drug use (Lipton, 1997). In 1999, a clinic-epidemiologic study revealed that sumatriptans could lead to overuse headache but lower than ergotamine derivative (Evers et al., 1999). In 2003, Patient with overuse headache transformed to chronic migraine was reported in 26.1% of drugs containing ergotamine derivatives, while the overuse of simple analgesics had 75.2% and triptans in 15.5% (Krymchantowski, 2003). Based on these evidences, it can be implied that the receptor of ergotamine might be specific site of the drug overuse in MOH.

CHAPTER III

MATERIALS AND METHODS

1. Materials

A. Drugs and antibodies

Sodium pentobarbital (Ceva Sante Animale, France)

Dihydroergotamine (DHE) (Tocris Bioscience, UK)

Dimethyl sulfoxide (DMSO)(Sigma-Aldrich, MO, USA)

Mouse anti-c-Fos monoclonal antibody (Calbiochem, Germany)

Mouse anti-β-actin monoclonal antibody (Sigma-Aldrich, MO, USA)

Anti-mouse HRP conjugated secondary antibody (Sigma-Aldrich, MO, USA)

B. Chemicals

Potassium chloride (KCl) (Merck, Germany)

BCA protein assay kit (Thermo Scientific, IL, USA)

Chemiluminescent kit (Thermo Scientific, IL, USA)

100x protease inhibitor (Cell Signaling Technology, MA, USA)

10x RIPA buffer (Cell Signaling Technology, MA, USA)

30% acrylamide (Applichem, MO, USA)

Page Ruler Prestained Protein Ladder, 10 to 170 kDa (Thermo Scientific, IL, USA)

TEMED (Bio-rad, UK)

Ammonium persulfate (APS) (Bio-rad, UK)

Bovine serum albumin (BSA) (Calbiochem, Germany)

Sodium dodecyl sulfate (SDS) (Bio-rad, UK)

Methanol (Merck, Germany)

Skim Milk Power (Sigma-Aldrich, USA)

Tris (Vivantis Technologies, Malaysia)

Glycine (Applichem, MO, USA)

Developer & Fixer (Kodak, UK)

C. Materials

Capillary glass (Sutter Instrument, CA, USA)

IV catherter 24G*3/4” (Nipro Corporation, Japan)

Needles and syringes (Nipro Corporation, Japan)

PVDF membrane (GE Healthcare Life Sciences, UK)

Amersham Hyperfilm ECL (GE Healthcare Life Sciences, UK)

D. Research instruments

Steriotaxic apparatus (Narishige, Japan)

Dental drill (NSK, Japan)

Hydraulic micromanipulator (Narishige, Japan)

MP100 data acquisition system (Biopac System Inc., CA, USA)

Microelectrode amplifier (Nihon Kohden, Japan)

Micropipette puller (Sutter Instrument, CA, USA)

Light microscope (Olympus Corporation, Japan)

Mini-PROTEAN Tetra Cell (Bio-rad, UK)

Mini Trans-Blot Module (Bio-rad, UK)

Power Pac HC Power Supply (Bio-rad, UK)

2. Experimental designs

In order to investigate the effect of dihydroergotamine (DHE) administration that affects cortical excitability and trigeminal nociception, the study was separated into 2 major experiments as followed:

EXPERIMENT I: The study of acute DHE exposure on the trigeminal nociception induced by CSD.

- CSD was continuously monitored for 2 hours. After the 3rd depolarization wave of CSD was presented, the rats were intravenously treated with 100 µg/kg of DHE into right femoral vein. The 0.1% DMSO in saline was used in control group.

- After the DC shift recording, the expression of c-Fos protein was examined in TNC by western blot analysis.

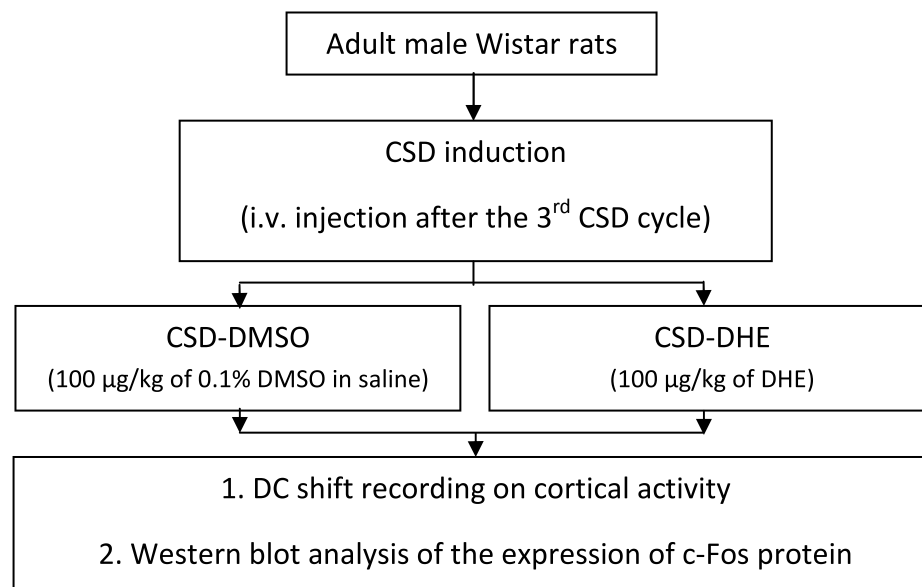

Figure 3.1 Diagram of the experimental design of the experiment I

EXPERIMENT II: The study of chronic DHE exposure on the trigeminal nociception induced by CSD.

- The rats were intraperitoneally treated with 100 µg/kg of DHE once-daily for 0-, 7-, 14-, and 28-day treatment. The 0.1% DMSO in saline was used in each control group. CSD was induced after 30 minutes of the last administration of 0.1% DMSO or DHE and continuously monitored for 1 hour.

- After the DC shift recording, the expression of c-Fos protein was examined in TNC by western blot analysis.

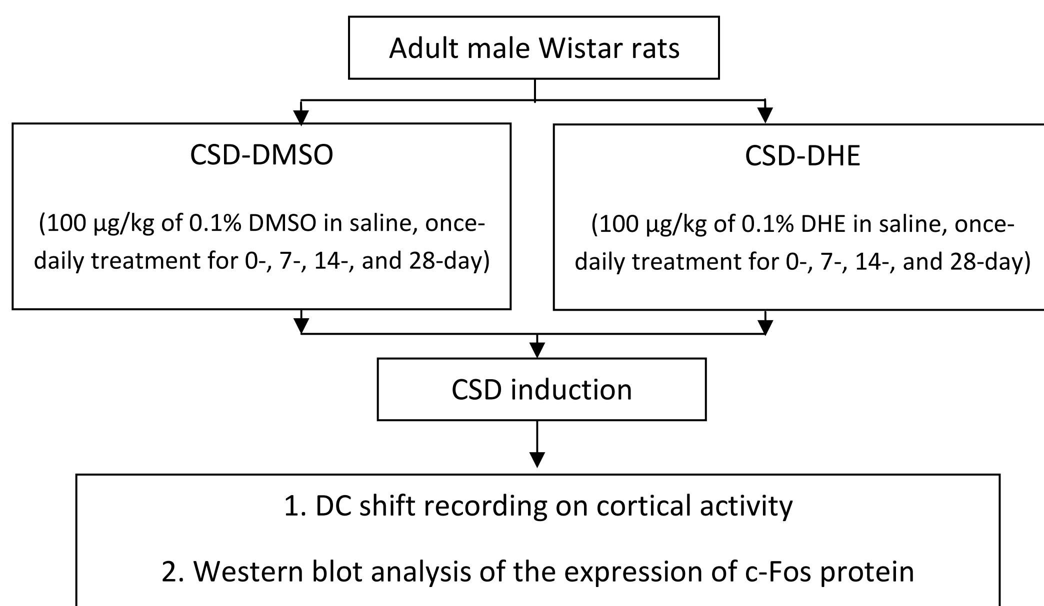

Figure 3.2 Diagram of the experimental design of the experiment II

3. Methods

Adult male Wistar rats weighing 250-350 grams were used in all experiments and purchased from the National Laboratory Animal Center, Mahidol University, Nakorn-Pathom, Thailand. Rats were housed in stainless cages in the ventilation room under a 12-hour dark-light cycle and allowed to food and water ad libitum. All of the protocols were approved by the Animal Care and Use Committee of Faculty of Medicine, Chulalongkorn University, Thailand.

A. Animal preparation

Rats were anesthetized by intraperitoneal injection of 60 mg/kg of body weight sodium pentobarbital. The level of anesthesia was closely controlled through the experiment and tested by lack of responsiveness to a tail pinch. A tracheotomy was performed for ventilation. Cannulation of the right femoral vein was performed for intravenous infusion of 0.9% normal saline and drugs.

B. Drugs administration

DHE was dissolved in 0.1% DMSO at 100 mg/ml, and diluted in normal saline (100 µg/ml of DHE). The control group was given the 0.1% DMSO as a vehicle in the same volume and condition. DHE administration was given to rats as followed:

In experiment I, an acute DHE exposure group, rats were received with 100 µg/kg of DHE, i.v., after the 3rd depolarization wave of CSD was presented.

In experiment II, a chronic DHE exposure group, rats were received with a once daily of 100 µg/kg of DHE for 0, 7, 14, and 28 days. The last administration of 100 µg/kg of DHE was performed at 30 minutes before CSD induction.

C. Surgical procedures

After animal preparation, a rat was placed and fixed the head in a steriotaxic frame. Skin and connective tissues were then removed to allow a craniotomy. Two craniotomies (each 2 mm in diameter) were prepared using a dental drill. The first hole was performed on frontal bone at 1 mm anterior from bregma and laterally from midline for inserting the glass capillary microelectrode. The second hole was performed on parietal bone at 7 mm posterior from bregma and 1 mm laterally from midline for CSD induction. Dura mater was carefully opened using 26 ½ G needle to expose the cortical surface. Normal saline was used for clean the hole and reduce the heat.

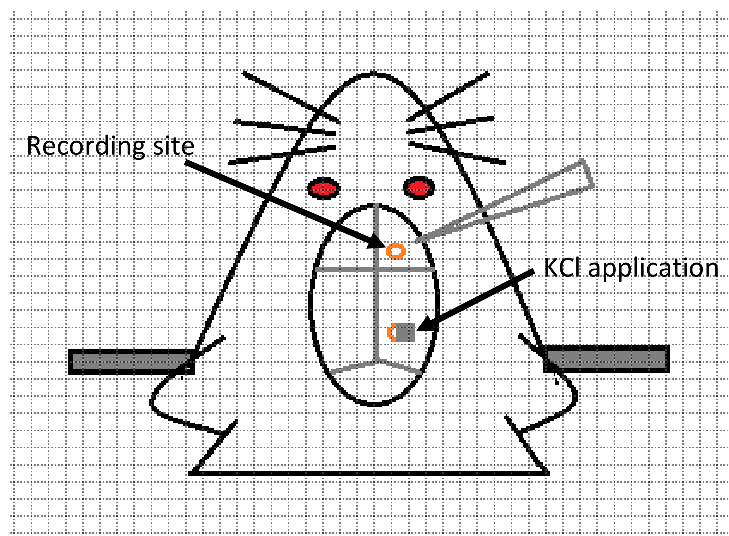

Figure 3.3 Craniotomy showing the recording site and location of KCl application.

D. Recording of extracellular potentials

A glass capillary microelectrode, which filled with 4M NaCl, was inserted into the cortex at 500 µm depth using the hydraulic micromanipulator. An Ag/AgCl was placed on the rat skin as a reference. A series of CSD waves was induced by topical

application of 3 mg of solid KCl. Electrocorticogram was recorded for 2 hours after acute drug administration and 1 hour after the last chronic drug administration.

Data analysis and interpretation

The measurement of CSD variables including the number of waves within 1 hour and 2 hours (waves), amplitude (the signal between baseline and maximum of each peak, mV), duration (time interval between the start to the end of each waveform, second), inter-peak latency (time interval between the maximum of 2 peaks, second), and area under the curve (the whole area of each waveform, mV.second) were analyzed in each waveform of cortical depolarization wave of CSD by AcqKnowledge 3.7.3, Biopac system.

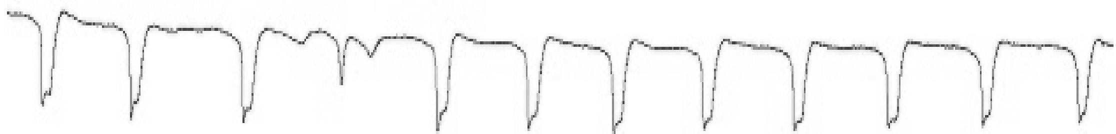

Figure 3.4 A series of cortical depolarization waves of CSD showing the pattern of waveform and the number of waves.

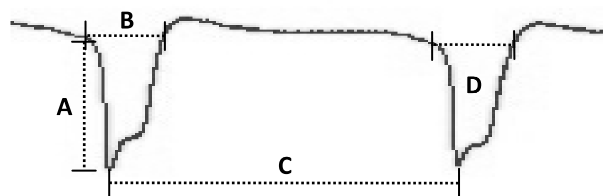

Figure 3.5 The measurement of CSD variables. A: amplitude, B: duration, C: inter-peak latency, D: area under the curve.

E. Western blot analysis

Tissue preparation

After recording of electrocorticogram, rats were euthanized by excessive dose of sodium pentobarbital. A right caudal medulla, which containing TNC (1-2 mm above obex to 5-7 mm below obex), was isolated in a block and washed in cool-saline before immediate immersing in liquid nitrogen.

Extraction of protein

A block of spinal cord containing TNC was washed in ice-cold tris buffer, pH 7.4 and homogenized in mixing of 1x RIPA buffer and 100x protease inhibitor cocktail with 21 G needle and sonicator at 32% amplitude. Insoluble materials were separated by centrifuged at 12,000 rpm for 15 minutes at 4°C. Supernatant was removed and stored at -80°C.

Total protein concentration measurement

Protein concentrations were quantified using BCA protein assay kit and measured spectrophotometer at 570 nm by micro-plate reader.

Western blotting analysis

The 30 μg of loading protein concentration were denatured at 95°C, 10 minutes, and separated by SDS-PAGE electrophoresis at 100 volts for 120 minutes, in 7.5% gel. After electrophoresis, gel containing protein was transferred to PVDF membrane at 0.35 amperes for 90 minutes.

After blotting, a PVDF membrane was washed with TBS and incubated in 5% non-fat milk in TBST at 4°C overnight for blocking non-specific. Membrane was then incubated in mouse anti-c-Fos monoclonal antibody, dilution 1:1000 in 3% BSA in TBST at room temperature for 3 hours. After primary antibody binding, membrane was washed with TBST 3 times (10 minutes each) and then incubated in anti-mouse horseradish peroxidase conjugated secondary antibody, dilution 1:10,000 in 5% BSA

in TBST. Membrane was repeatedly washed with TBST and moved into TBS solution before c-Fos protein band detection.

The visualization of c-Fos protein band was detected after repeated washing membrane in TBST using ECL kit for 5 minutes in dark room. Membrane was placed on the plastic sheet, covered with film cassette, and exposed to hyper-film for various the suitable times.

Data analysis and interpretation

Exposure film with c-Fos protein band was scanned by Epson printer (Epson Stylus TX220). Density of c-Fos protein band at 55 kDa were measured on a densitometer (Specify which one) and interpreted using a Scion Image Corporation software. Signals from blotting were normalized to β-actin at 43 kDa in the same blot.

F. Statistical analysis

All data were presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics data editor. An independent sample *t*-test was used to determine differences between CSD-DMSO and CSD-DHE group. A one-way ANOVA (Bonferroni post hoc test) was used to compare the data among the other groups. A probability value of less than 0.05 was considered to be statistically significant.

CHAPTER IV

RESULTS

In order to investigate the role of dihydroergotamine (DHE) administration that affects cortical excitability and trigeminal nociception, the experiment were divided into 2 parts as followed:

PART I: Effect of acute DHE exposure on cortical activity and CSD-induced trigeminal nociception.

PART II: Effect of chronic DHE exposure on cortical activity and CSD-induced trigeminal nociception.

Each part were subdivided into 2 parts; DHE administration on cortical activity indicated by CSD development and CSD-evoked trigeminal nociception indicated by c-Fos expression in brain stem containing trigeminal nucleus caudalis (TNC).

PART I:

Effect of acute DHE exposure on cortical activity and CSD-induced trigeminal nociception

A. Effect of acute DHE administration on the cortical activity

The change of direct current (DC) shift was observed to evaluate the CSD development. A series of CSD waves was generated using solid KCl application on parietal cortex within the 2-hour recording time (Figure 4.1). The measurement of CSD variables included the number of wave, amplitude, peak duration, inter-peak latency, and area under the curve (AUC). DMSO was used as the control of drug injection.

In acute CSD-DHE group and CSD-DMSO group, the mean number of CSD waves was 18.36 ± 3.30 and 18.14 ± 5.76, respectively, the CSD amplitude was 29.85 ± 3.45 and 28.88 ± 2.94 mV, respectively, the mean peak duration was 60.07 ± 14.65 and 61.14 ± 8.33 seconds, respectively, the mean inter-peak latency was 336.89 ± 89.92 and 348.40 ± 83.95 seconds, respectively, and the sum of AUC was 61.31 ± 11.64 and 52.54 ± 12.05 mV-second.

Comparing between acute CSD-DHE and CSD-DMSO group, there was no significant difference was observed in all variables (Table 4.1).

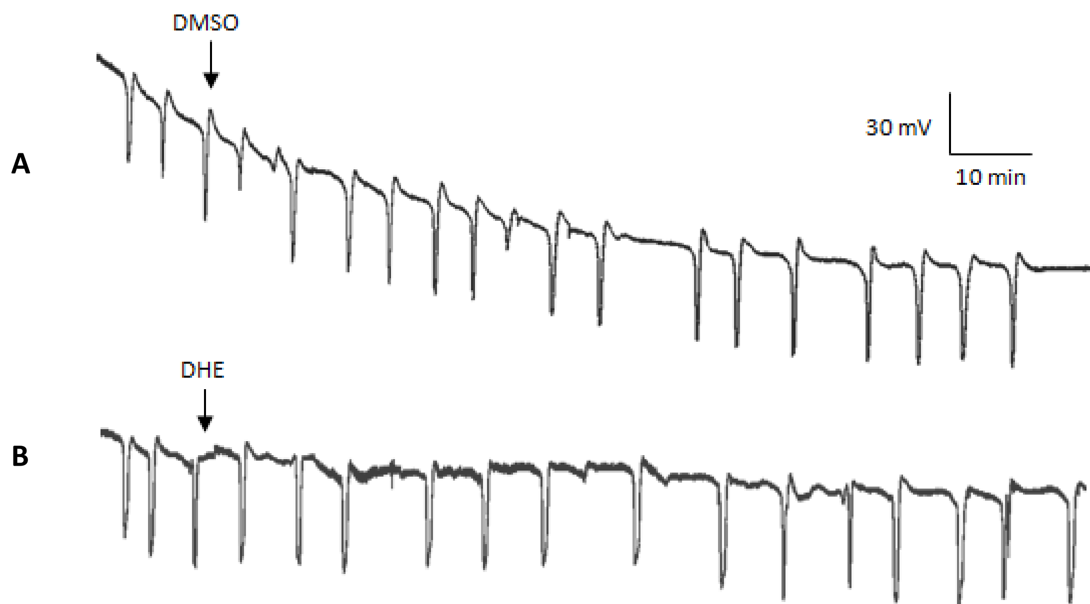

Figure 4.1 Representative tracing shows the effect of acute DHE administration on CSD waves within 2 hours recording time. The arrow indicates intravenous administration of DMSO (0.1% in saline) or DHE after the third CSD cycle. A: control (DMSO). B: acute DHE administration.

Table 4.1 The measurement of CSD variables within 2 hours recording time between acute CSD-DMSO and CSD-DHE group.

Variable measurement	Acute CSD-DMSO (n=9)	Acute CSD-DHE (n=14)	*p*-value
Number of waves/2 hours	18.14 ± 5.76	18.36 ± 3.30	0.906
Amplitude (mV)	28.88 ± 2.94	29.85 ± 3.45	0.532
Peak duration (seconds)	61.14 ± 8.33	60.07 ± 14.65	0.847
Inter-peak latency (seconds)	348.40 ± 83.95	336.89 ± 89.92	0.786
AUC (mV-second)	52.54 ± 12.05	61.31 ± 11.64	0.137

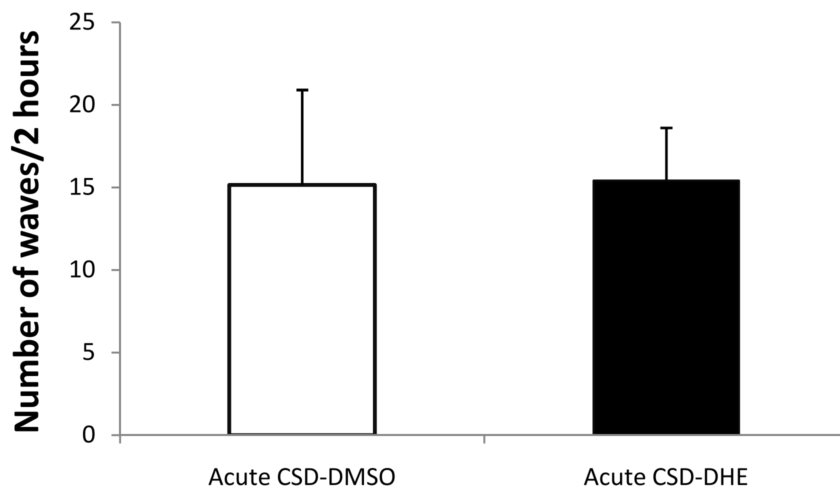

Figure 4.2 Mean and SD of the number of waves in between CSD-DMSO and CSD-DHE group in acute administration.

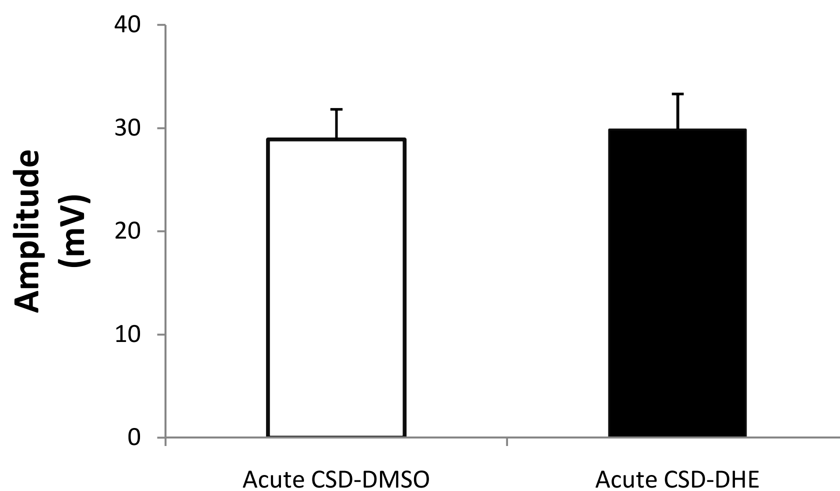

Figure 4.3 Mean and SD of the amplitude in CSD-DMSO and CSD-DHE group in acute administration.

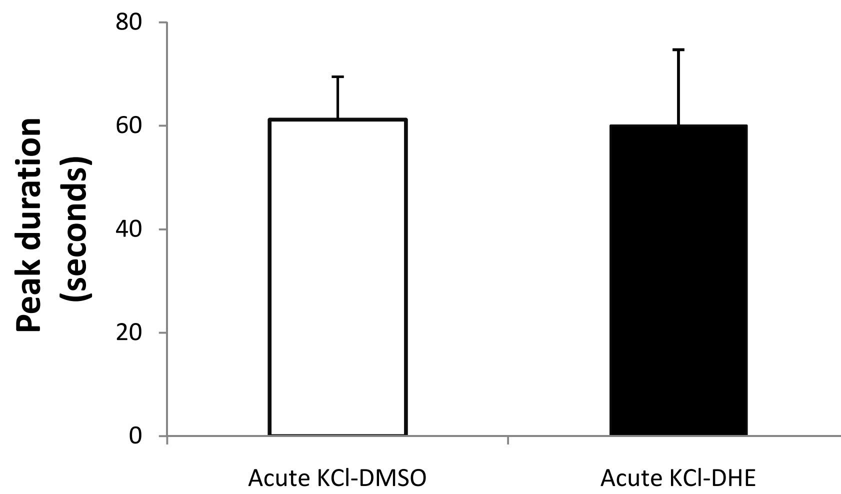

Figure 4.4 Mean and SD of the peak duration in CSD-DMSO and CSD-DHE group in acute administration.

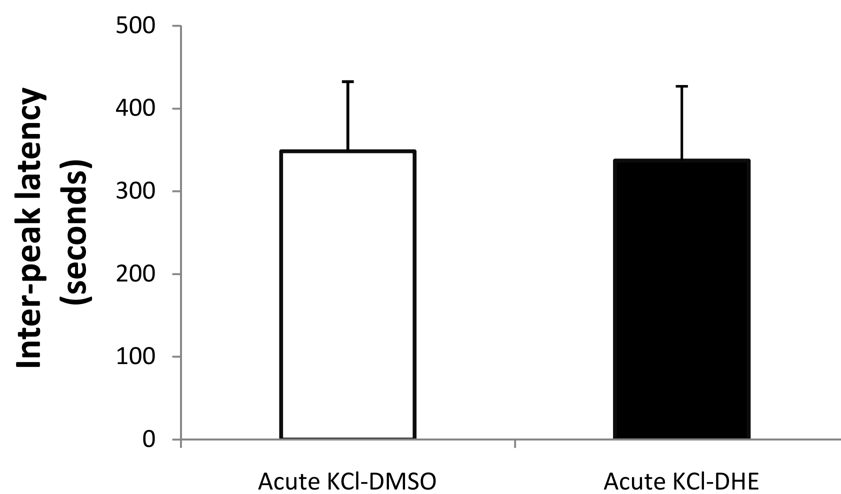

Figure 4.5 Mean and SD of the inter-peak latency in CSD-DMSO and CSD-DHE group in acute administration.

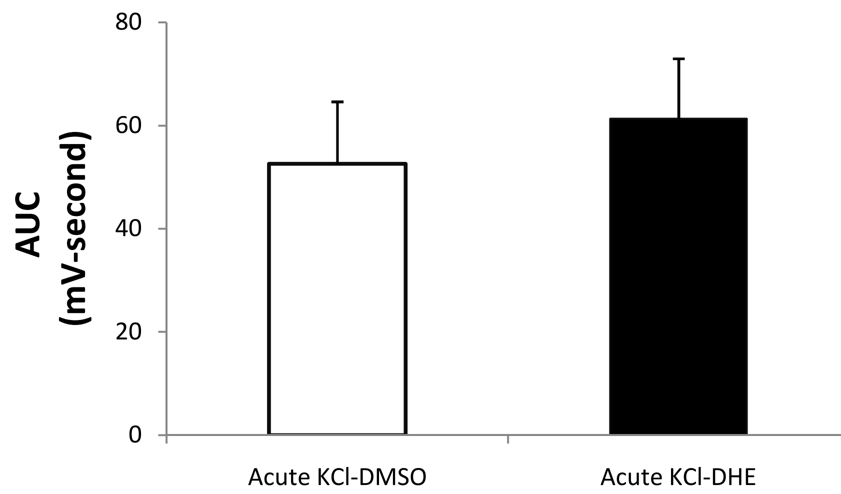

Figure 4.6 Mean and SD of the AUC in CSD-DMSO and CSD-DHE group in acute administration.

B. Effect of acute DHE administration on trigeminal nociception

The expression of c-Fos was used as a marker indicating the neuronal activation of second order neurons in trigeminal system. By using Western blot analysis, the expression of c-Fos protein was quantified as to provide a relative density of β-actin (Figure 4.7).

The result showed that acute CSD-DHE significantly decreased the c-Fos protein expression comparing with CSD-DMSO group. Relative density of c-Fos/β-actin was 0.61 ± 0.02 in CSD-DHE group and 0.70 ± 0.07 in CSD-DMSO group (*p* = 0.037, table 4.2).

Table 4.2 The relative density of c-Fos and β-actin between acute CSD-DMSO and CSD-DHE group. (**p*<0.05, Student's *t*-test compared with their control group)

Acute CSD-DMSO (n=5)	Acute CSD-DHE (n=5)	*p*-value
0.70 ± 0.07	0.61 ± 0.02	0.037*

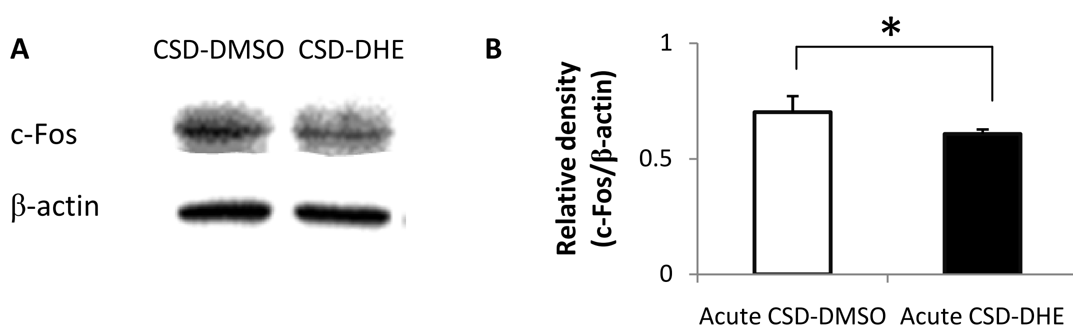

Figure 4.7 The expression of c-Fos protein in TNC in acute CSD-DMSO and acute CSD-DHE group detected by western blot analysis. (*p<0.05, Student's *t*-test)

PART II:

Effect of chronic DHE exposure on cortical activity and CSD-induced trigeminal nociception

A. Effect of chronic DHE administration for various days on the cortical activity

The change of DC shift was observed to evaluate the CSD development. A series of CSD waves was generated using solid KCl application on parietal cortex within the 1-hour recording time (Figure 4.8). The measurement of CSD variables included the number of wave, amplitude, peak duration, inter-peak latency, and AUC. The result of this part was classified as followed by the CSD variables. (Table 4.3)

- Effect of chronic DHE administration on the number of waves of CSD development

In chronic CSD-DHE group and CSD-DMSO group, the mean number of CSD waves was 14.33 ± 2.57 and 12.67 ± 3.08 waves for 0-day administration, respectively, 12.78 ± 2.86 and 14.25 ± 2.19 waves for 7-day administration, respectively, 12.08 ± 2.61 and 12.71 ± 3.09 waves for 14-day administration, respectively, 11.63 ± 2.90 and 13.13 ± 2.85 waves for 28-day administration, respectively.

- Effect of chronic DHE administration on the amplitude of CSD development

In chronic CSD-DHE group and CSD-DMSO group, the amplitude was 27.88 ± 2.44 and 26.67 ± 3.88 mV for 0-day administration, respectively, 28.23 ± 3.50 and 26.16 ± 4.23 mV for 7-day administration, respectively, 28.75 ± 3.76 and 27.69 ± 3.83 mV for 14-day administration, respectively, 29.69 ± 2.07 and 26.34 ± 3.48 mV for 28-day administration, respectively.

- *Effect of chronic DHE administration on the peak duration of CSD development*

In chronic CSD-DHE group and CSD-DMSO group, the peak duration was 52.73 ± 8.52 and 57.85 ± 12.13 seconds for 0-day administration, respectively, 54.57 ± 7.43 and 55.23 ± 5.48 seconds for 7-day administration, respectively, 50.09 ± 8.51 and 52.15 ± 4.84 seconds for 14-day administration, respectively, 57.64 ± 16.41 and 58.90 ± 5.62 seconds for 28-day administration, respectively.

- *Effect of chronic DHE administration on the inter-peak latency of CSD development*

In chronic CSD-DHE group and CSD-DMSO group, the inter-peak latency was 245.26 ± 21.03 and 282.51 ± 57.41 seconds for 0-day administration, respectively, 279.92 ± 64.75 and 265.64 ± 55.45 seconds for 7-day administration, respectively, 292.05 ± 59.91 and 274.88 ± 43.41 seconds for 14-day administration, respectively, 286.09 ± 55.97 and 271.88 ± 56.49 seconds for 28-day administration, respectively.

- *Effect of chronic DHE administration on the AUC of CSD development*

In chronic CSD-DHE group and CSD-DMSO group, the AUC was 41.72 ± 7.13 and 43.78 ± 9.97 mV.s for 0-day administration, respectively, 42.43 ± 8.55 and 39.41 ± 9.18 mV.s for 7-day administration, respectively, 47.22 ± 6.63 and 38.06 ± 6.03 mV.s for 14-day administration, respectively, 52.15 ± 11.02 and 39.46 ± 5.65 mV.s for 28-day administration, respectively.

According to the above results, the sum of AUC significantly increased within 1-hour recording time in 14- and 28- day chronic administration of CSD-DHE group compared with CSD-DMSO group. However, there was no significant difference in other measurement variables between CSD-DHE and CSD-DMSO group for each day chronic administration.

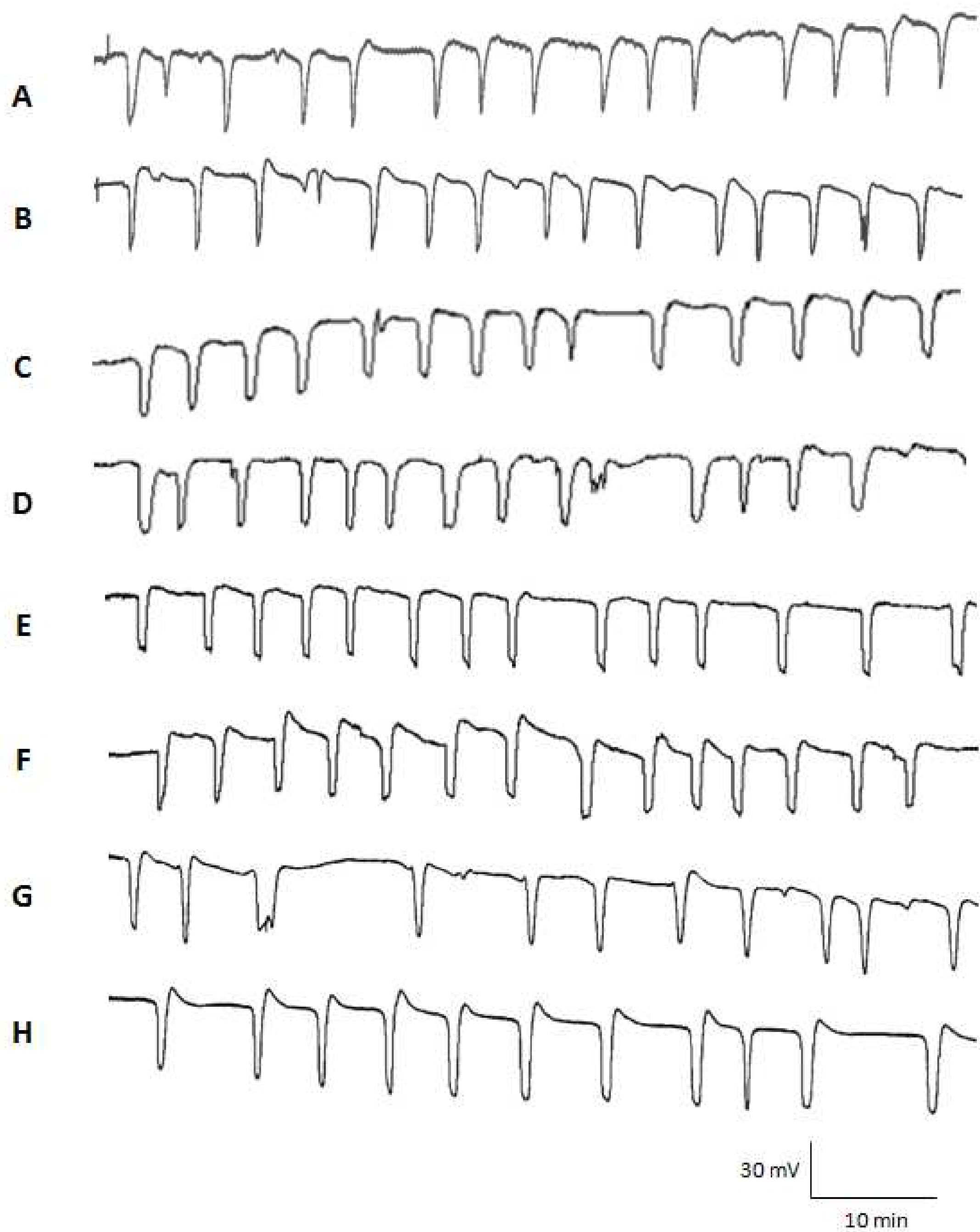

Figure 4.8 Representative tracing shows the effect of chronic DHE administration on CSD waves for 1-hour recording time. A: 0-day DMSO, B: 0-day DHE, C: 7-day DMSO, D: 7-day DHE**,** E: 14-day DMSO, F: 14-day DHE, G: 28-day DMSO, H: 28-day DHE.

Table 4.3 The measurement of CSD variables within 1-hour recording time between chronic CSD-DMSO and CSD-DHE group for various days' administration. (Bold text shows *p*-value between CSD-DMSO and CSD-DHE group for each day administration, *$p<0.05$ Student's *t*-test compared with their control group)

<table>
<tr><th colspan="2">Variable measurement</th><th colspan="2">Number of waves</th><th colspan="2">Amplitude (mV)</th><th colspan="2">Peak duration (seconds)</th><th colspan="2">Inter-peak latency (seconds)</th><th colspan="2">AUC (mV-second)</th></tr>
<tr><td rowspan="2">Chronic 0-day</td><td>CSD-DMSO (n=9)</td><td>12.67 ± 3.08</td><td rowspan="2">0.193</td><td>26.67 ± 3.88</td><td rowspan="2">0.394</td><td>57.85 ± 12.13</td><td rowspan="2">0.294</td><td>282.51 ± 57.41</td><td rowspan="2">0.080</td><td>43.78 ± 9.97</td><td rowspan="2">0.669</td></tr>
<tr><td>CSD-DHE (n=11)</td><td>14.33 ± 2.57</td><td>27.88 ± 2.44</td><td>52.73 ± 8.52</td><td>245.26 ± 21.03</td><td>41.72 ± 7.13</td></tr>
<tr><td rowspan="2">Chronic 7-day</td><td>CSD-DMSO (n=8)</td><td>14.25 ± 2.19</td><td rowspan="2">0.257</td><td>26.16 ± 4.23</td><td rowspan="2">0.296</td><td>55.23 ± 5.48</td><td rowspan="2">0.858</td><td>265.64 ± 55.45</td><td rowspan="2">0.635</td><td>39.41 ± 9.18</td><td rowspan="2">0.493</td></tr>
<tr><td>CSD-DHE (n=11)</td><td>12.78 ± 2.86</td><td>28.23 ± 3.50</td><td>54.57 ± 7.43</td><td>279.92 ± 64.75</td><td>42.43 ± 8.55</td></tr>
<tr><td rowspan="2">Chronic 14-day</td><td>CSD-DMSO (n=7)</td><td>12.71 ± 3.09</td><td rowspan="2">0.640</td><td>27.69 ± 3.83</td><td rowspan="2">0.566</td><td>52.15 ± 4.84</td><td rowspan="2">0.602</td><td>274.88 ± 43.41</td><td rowspan="2">0.581</td><td>38.06 ± 6.03</td><td rowspan="2">0.034*</td></tr>
<tr><td>CSD-DHE (n=12)</td><td>12.08 ± 2.61</td><td>28.75 ± 3.76</td><td>50.09 ± 8.51</td><td>292.05 ± 59.91</td><td>47.22 ± 6.63</td></tr>
<tr><td rowspan="2">Chronic 28-day</td><td>CSD-DMSO (n=8)</td><td>13.13 ± 2.85</td><td rowspan="2">0.356</td><td>26.34 ± 3.48</td><td rowspan="2">0.258</td><td>58.90 ± 5.62</td><td rowspan="2">0.848</td><td>271.88 ± 56.49</td><td rowspan="2">0.984</td><td>39.46 ± 5.65</td><td rowspan="2">0.036*</td></tr>
<tr><td>CSD-DHE (n=16)</td><td>11.63 ± 2.90</td><td>29.69 ± 2.07</td><td>57.64 ± 16.41</td><td>286.09 ± 55.97</td><td>52.15 ± 11.02</td></tr>
</table>

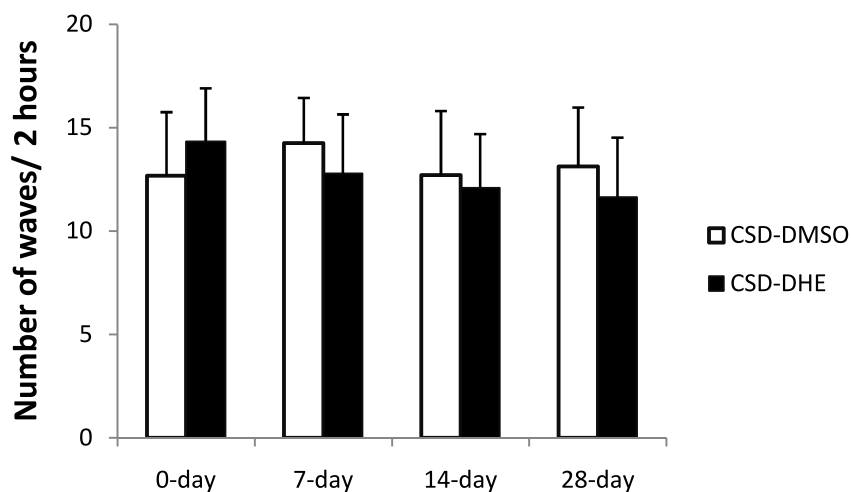

Figure 4.9 Mean and SD of the number of waves in CSD-DMSO and CSD-DHE group in chronic 0-day, 7-day, 14-day, and 28-day administration.

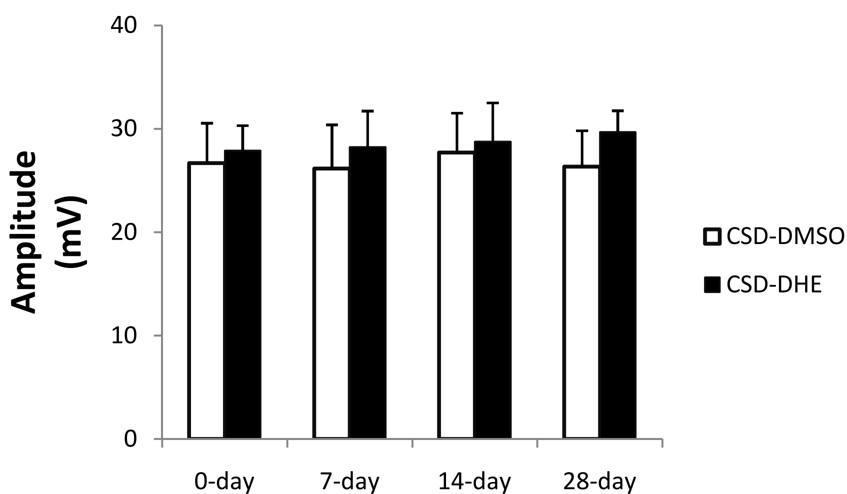

Figure 4.10 Mean and SD of the amplitude in CSD-DMSO and CSD-DHE group in chronic 0-day, 7-day, 14-day, and 28-day administration.

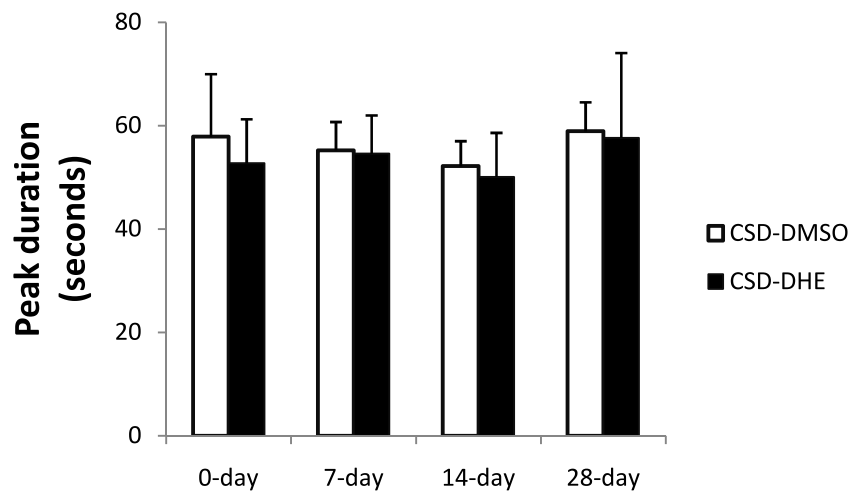

Figure 4.11 Mean and SD of the peak duration in CSD-DMSO and CSD-DHE group in chronic 0-day, 7-day, 14-day, and 28-day administration. (*p<0.05, Student's t-test compared with their control group)

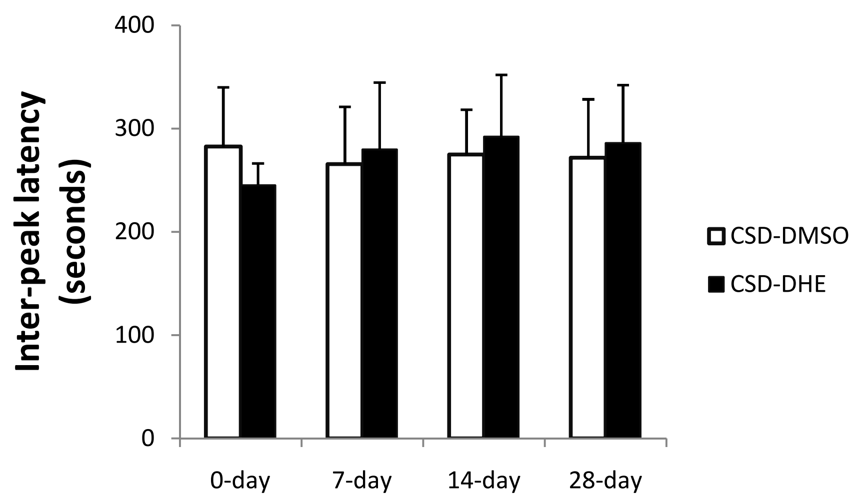

Figure 4.12 Mean and SD of the inter-peak latency in CSD-DMSO and CSD-DHE group in chronic 0-day, 7-day, 14-day, and 28-day administration.

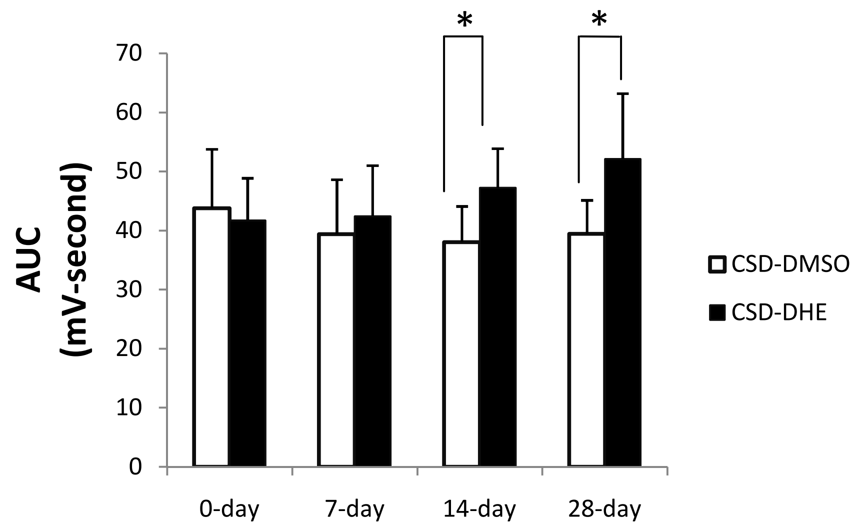

Figure 4.13 Mean and SD of the AUC in CSD-DMSO and CSD-DHE group in chronic 0-day, 7-day, 14-day, and 28-day administration. (*p<0.05, Student's *t*-test compared with their control group)

B. Effect of chronic DHE administration for various days on trigeminal nociception

In chronic CSD-DHE and CSD-DMSO group, relative density of c-Fos/β-actin was 0.48 ± 0.10 and 0.62 ± 0.08 for 0-day administration, respectively, 0.56 ± 0.10 and 0.49 ± 0.11 for 7-day administration, respectively, 0.85 ± 0.13 and 0.61 ± 0.04 for 14-day administration, respectively, 0.79 ± 0.13 and 0.59 ± 0.06 for 28-day administration, respectively.

The result showed the significant altering in the expression of c-Fos between chronic CSD-DHE and CSD-DMSO group for 0-, 14-, and 28-day administration (p = 0.043, 0.020, and 0.022), respectively. By contrast, there was no significant change in the expression of c-Fos for 7-day administration. There was not any significant difference tested by one-way ANOVA. (Table 4.4)

Table 4.4 The relative density of c-Fos and β-actin between CSD-DMSO and CSD-DHE group for chronic 0-day, 7-day, 14-day, and 28-day administration. (*p<0.05, Student's t-test compared with their control group)

Relative density c-Fos/β-actin	CSD-DMSO	CSD-DHE	*p*-value
0-day administration	0.62 ± 0.08 (n=5)	0.48 ± 0.10 (n=5)	0.043*
7-day administration	0.56 ± 0.10 (n=4)	0.49 ± 0.11 (n=5)	0.469
14-day administration	0.61 ± 0.04 (n=3)	0.85 ± 0.13 (n=6)	0.020*
28-day administration	0.59 ± 0.06 (n=4)	0.79 ± 0.13 (n=7)	0.022*

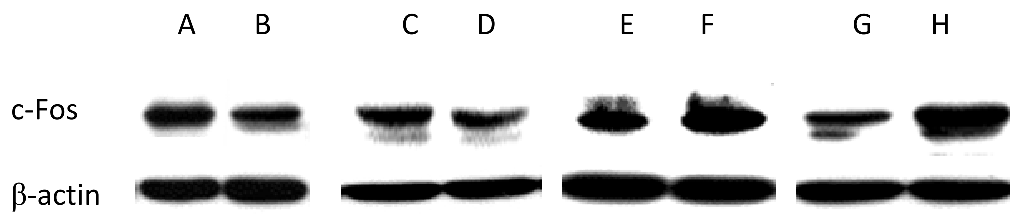

Figure 4.14 The expression of c-Fos protein in TNC between chronic CSD-DMSO and CSD-DHE group detected by western blot analysis. A: 0-day DMSO, B: 0-day DHE, C: 7-day DMSO, D: 7-day DHE**,** E: 14-day DMSO, F: 14-day DHE, G: 28-day DMSO, H: 28-day DHE.

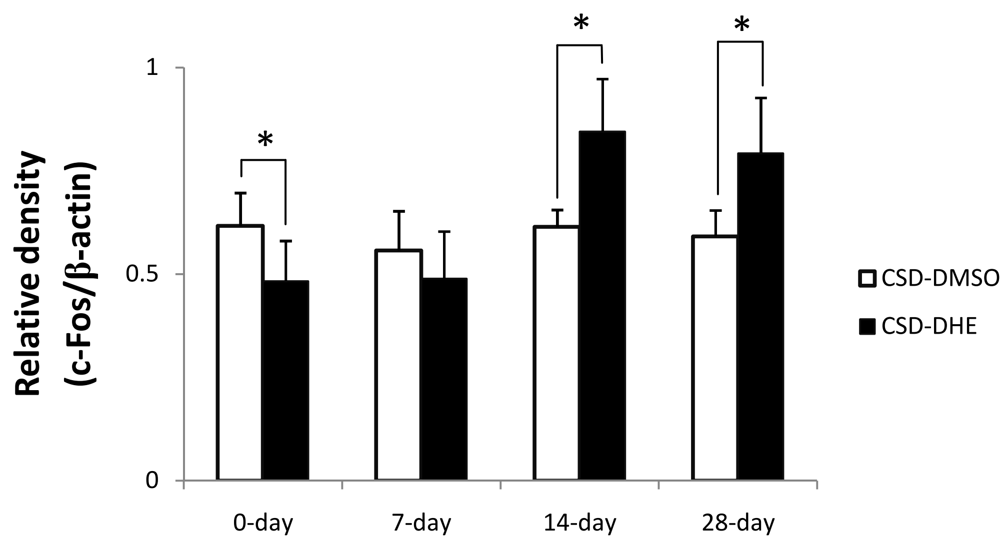

Figure 4.15 Comparing of a relative density of c-Fos/β-actin in TNC between CSD-DMSO and CSD-DHE group in chronic 0-day, 7-day, 14-day, and 28-day administration. (*p<0.05, Student's t-test compared with their control group)

CHAPTER V

DISCUSSION

The experiments of the present study were proceeded to examine the effects of acute and chronic DHE administration that contribute to cortical excitability and trigeminal nociception.

Effect of acute DHE administration on CSD-induced trigeminal nociception

In acute DHE administration, the results have shown that DHE can attenuate c-Fos protein expression in TNC without any alterations of CSD variables. The CSD variables were used to indicate cortical neuron activity.

A reduction of trigeminal nucleus caudalis c-Fos expression is correlated to the decrease in trigeminal activation and nociception. This hypothesis is consistent with antimigraine effect of acute DHE administration. The effects of DHE are more prominent in peripheral sites such as cranial vessels. Numerous binding receptors such as serotoninergic, adrenergic and dopaminergic receptors are proven to be functional for DHE causing vasoconstriction effect and a decrease in neuropeptide mediators released from perivascular nerves (Saper and Silberstein, 2006). Thus, DHE may modulate trigeminal nociception but does not modify cortical activity.

Effect of chronic DHE administration on CSD-induced trigeminal nociception

In this study, chronic exposures of DHE (14 and 28 days) altered cortical activity by increasing AUC of CSD waveforms whereas 0 and 7 days exposures had no effect. The alteration of CSD was parallel with the change of c-Fos expression in the brainstem that c-Fos expression did not change after chronic exposure of DHE 0 and 7 days but it increased within 14 to 28 days. These findings indicate that chronic administration of DHE can enhance cortical excitability and in trigeminal nociception. Several studies have also reported that long-term usage of ergot alkaloids can contribute to MOH (Lipton, 1997; Evers et al., 1999).

One possible mechanism of chronic DHE administration-induced MOH involves the alteration in cortical hyperexcitability (Bongsebandhu-phubhakdi and Srikiatkhachorn, 2012). In this study, the changes of the AUC of CSD waveforms were observed whereas those of other CSD variables including the number of waves, amplitude, and inter-peak latency were unaltered. The change in AUC after chronic DHE administration is due to the repolarization phase of CSD. This increasing pattern of AUC was similar to the study of serotonin depletion (Supornsilpchai et al., 2006). Moreover, chronic paracetamol exposure has shown that there was a change in the number of waves but not in AUC (Supornsilpchai et al., 2010). These results in chronic paracetamol exposure are entirely opposite to the present study that there was an increase in CSD waveforms but no change in the summation of AUC by DHE exposure. These inconsistency between chronic paracetamol and DHE is still underinvestigated yet may be dued to different pathophysiology. Additionally, an increase in c-Fos protein expression within the brainstem was also observed in this study. The change in trigeminal nociceptive system is also implicated to be one of underlying mechanisms of MOH. It is explained that chronic medication has an ability to develop the change in trigeminal afferent pathway (De Felice and Porreca, 2009). An upregulation of some neuropeptides have also been detected at peripheral sites after chronic triptans treatment leading to the latent sensitization which results in

MOH (De Felice et al., 2010). Another proposed explanation of MOH also involves a derangement of central modulating system (Bongsebandhu-phubhakdi and Srikiatkhachorn, 2012). Dysfunction of this system may promote nociception and increase of cortical excitability in MOH patients. However, more evidence are required to establish a correlation between DHE and MOH.

CHAPTER VI

CONCLUSION

The present study demonstrated that acute and chronic administration of DHE can modulate CSD-induced trigeminal nociception. The acute DHE administration during migraine attack can attenuate the expression of c-Fos protein in caudal brainstem containing TNC without any changes in CSD development. On the contrary, chronic treatment with DHE has significantly changed the AUC of CSD waveforms in accordance with increase of c-Fos expression in the TNC. These findings indicate that DHE administration decrease trigeminal nociception in acute phase, while chronic DHE administration increase cortical excitability and trigeminal nociception.

REFERENCES

Ahmed, F., R. Parthasarathy, and M. Khalil. Chronic Daily Headaches. Ann Indian Acad Neurol 15 (2012) : S40-50.

Akerman, S., and P. J. Goadsby. The Role of Dopamine in a Model of Trigeminovascular Nociception. J Pharmacol Exp Ther 314,1 (2005) : 162-9.

Ayata, C., H. Jin, C. Kudo, T. Dalkara, and M. A. Moskowitz. Suppression of Cortical Spreading Depression in Migraine Prophylaxis. Ann Neurol 59,4 (2006) : 652-61.

Ayzenberg, I., M. Obermann, K. Leineweber, L. Franke, M. S. Yoon, H. C. Diener, and Z. Katsarava. Increased Activity of Serotonin Uptake in Platelets in Medication Overuse Headache Following Regular Intake of Analgesics and Triptans. J Headache Pain 9,2 (2008) : 109-12.

Bergerot, A., R. J. Storer, and P. J. Goadsby. Dopamine Inhibits Trigeminovascular Transmission in the Rat. Ann Neurol 61,3 (2007) : 251-62.

Bongsebandhu-Phubhakdi, S., T. Phisonkulkasem, and A. Srikiatkhachorn. Nociceptin/Orphanin Fq Modulates Cortical Activity and Trigeminal Nociception. Headache 51,8 (2011) : 1245-53.

Bongsebandhu-phubhakdi, S., and A. Srikiatkhachorn. Pathophysiology of Medication-Overuse Headache: Implications from Animal Studies. Curr Pain Headache Rep 16,1 (2012) : 110-5.

Burstein, R., D. Yarnitsky, I. Goor-Aryeh, B. J. Ransil, and Z. H. Bajwa. An Association between Migraine and Cutaneous Allodynia. Ann Neurol 47,5 (2000) : 614-24.

Casucci, G., V. Villani, and F. Frediani. Central Mechanism of Action of Antimigraine Prophylactic Drugs. Neurol Sci 29 (2008) : S123-6.

Chatchaisak, D., A. Srikiatkhachorn, S. Maneesri-le Grand, P. Govitrapong, and B. Chetsawang. The Role of Calcitonin Gene-Related Peptide on the Increase in Transient Receptor Potential Vanilloid-1 Levels in Trigeminal Ganglion and Trigeminal Nucleus Caudalis Activation of Rat. J Chem Neuroanat 47 (2013) : 50-6.

Cutrer, F. M., V. Limmroth, G. Ayata, and M. A. Moskowitz. Attenuation by Valproate of C-Fos Immunoreactivity in Trigeminal Nucleus Caudalis Induced by Intracisternal Capsaicin. Br J Pharmacol 116,8 (1995) : 3199-204.

Dahlof, C., and A. Maassen Van Den Brink. Dihydroergotamine, Ergotamine, Methysergide and Sumatriptan - Basic Science in Relation to Migraine Treatment. Headache 52,4 (2012) : 707-14.

De Felice, M., M. H. Ossipov, and F. Porreca. Update on Medication-Overuse Headache. Curr Pain Headache Rep 15,1 (2011) : 79-83.

De Felice, M., M. H. Ossipov, R. Wang, J. Lai, J. Chichorro, I. Meng, D. W. Dodick, T. W. Vanderah, G. Dussor, and F. Porreca. Triptan-Induced Latent Sensitization: A Possible Basis for Medication Overuse Headache. Ann Neurol 67,3 (2010) : 325-37.

De Felice, M., and F. Porreca. Opiate-Induced Persistent Pronociceptive Trigeminal Neural Adaptations: Potential Relevance to Opiate-Induced Medication Overuse Headache. Cephalalgia 29,12 (2009) : 1277-84.

Evers, S., I. Gralow, B. Bauer, B. Suhr, A. Buchheister, I. W. Husstedt, and E. B. Ringelstein. Sumatriptan and Ergotamine Overuse and Drug-Induced Headache: A Clinicoepidemiologic Study. Clin Neuropharmacol 22,4 (1999) : 201-6.

Holland, P. R., S. Akerman, and P. J. Goadsby. Cortical Spreading Depression-Associated Cerebral Blood Flow Changes Induced by Mechanical Stimulation Are Modulated by Ampa and Gaba Receptors. Cephalalgia 30,5 (2010) : 519-27.

Hoskin, K. L., H. Kaube, and P. J. Goadsby. Central Activation of the Trigeminovascular Pathway in the Cat Is Inhibited by Dihydroergotamine. A C-Fos and Electrophysiological Study. Brain 119 (1996) : 249-56.

Hoskin, K. L., H. Kaube, and P. J. Goadsby. Sumatriptan Can Inhibit Trigeminal Afferents by an Exclusively Neural Mechanism. Brain 119 (1996) : 1419-28.

Kaji, Y., and K. Hirata. Characteristics of Mood Disorders in Japanese Patients with Medication-Overuse Headache. Intern Med 48,12 (2009) : 981-6.

Kaube, H., and P. J. Goadsby. Anti-Migraine Compounds Fail to Modulate the Propagation of Cortical Spreading Depression in the Cat. Eur Neurol 34,1 (1994) : 30-5.

Krymchantowski, A. V. Overuse of Symptomatic Medications among Chronic (Transformed) Migraine Patients: Profile of Drug Consumption. Arq Neuropsiquiatr 61,1 (2003) : 43-7.

Lauritzen, M. Pathophysiology of the Migraine Aura. The Spreading Depression Theory. Brain 117 (1994) : 199-210.

Ligthart, L., M. M. Gerrits, D. I. Boomsma, and B. W. Penninx. Anxiety and Depression Are Associated with Migraine and Pain in General: An Investigation of the Interrelationships. J Pain (2013).

Lipton, R. B. Ergotamine Tartrate and Dihydroergotamine Mesylate: Safety Profiles. Headache 37 (1997) : S33-41.

Lynch, J. J., Jr., T. J. Detwiler, S. A. Kane, and C. P. Regan. Effect of Calcitonin Gene-Related Peptide Receptor Antagonism on the Systemic Blood Pressure Responses to Mechanistically Diverse Vasomodulators in Conscious Rats. J Cardiovasc Pharmacol 56,5 (2010) : 518-25.

Maneepak, M., S. le Grand, and A. Srikiatkhachorn. Serotonin Depletion Increases Nociception-Evoked Trigeminal Nmda Receptor Phosphorylation. Headache 49,3 (2009) : 375-82.

Marichal-Cancino, B. A., A. Gonzalez-Hernandez, G. Manrique-Maldonado, Salinas Ruiz, II, A. H. Altamirano-Espinoza, A. MaassenVanDenBrink, and C. M. Villalon. Intrathecal Dihydroergotamine Inhibits Capsaicin-Induced Vasodilatation in the Canine External Carotid Circulation Via Gr127935- and Rauwolscine-Sensitive Receptors. Eur J Pharmacol 692,1-3 (2012) : 69-77.

Markowitz, S., K. Saito, and M. A. Moskowitz. Neurogenically Mediated Plasma Extravasation in Dura Mater: Effect of Ergot Alkaloids. A Possible Mechanism of Action in Vascular Headache. Cephalalgia 8,2 (1988) : 83-91.

Masterson, C. G., and P. L. Durham. DHE Repression of ATP-Mediated Sensitization of Trigeminal Ganglion Neurons. Headache 50,9 (2010) : 1424-39.

Meng, I. D., D. Dodick, M. H. Ossipov, and F. Porreca. Pathophysiology of Medication Overuse Headache: Insights and Hypotheses from Preclinical Studies. Cephalalgia 31,7 (2011) : 851-60.

Merikangas, K. R. Contributions of Epidemiology to Our Understanding of Migraine. Headache 53,2 (2013) : 230-46.

Mitsikostas, D. D., and M. Sanchez del Rio. Receptor Systems Mediating C-Fos Expression within Trigeminal Nucleus Caudalis in Animal Models of Migraine. Brain Res Brain Res Rev 35,1 (2001) : 20-35.

Modi, S., and D. M. Lowder. Medications for Migraine Prophylaxis. Am Fam Physician 73,1 (2006) : 72-8.

Moreau, P. G., and P. Pichon. [Dihydroergotamine in the Treatment of Isolated Orthostatic Retinal Hypotension]. Bull Soc Ophtalmol Fr 67,5 (1967) : 482-9.

Moskowitz, M. A., K. Nozaki, and R. P. Kraig. Neocortical Spreading Depression Provokes the Expression of C-Fos Protein-Like Immunoreactivity within Trigeminal Nucleus Caudalis Via Trigeminovascular Mechanisms. J Neurosci 13,3 (1993) : 1167-77.

Nilsson, P., L. Hillered, Y. Olsson, M. J. Sheardown, and A. J. Hansen. Regional Changes in Interstitial K+ and Ca2+ Levels Following Cortical Compression Contusion Trauma in Rats. J Cereb Blood Flow Metab 13,2 (1993) : 183-92.

Perhoniemi, V., J. Vuorinen, P. Myllynen, A. Kivioja, and K. Lindevall. The Effect of Enoxaparin in Prevention of Deep Venous Thrombosis in Hip and Knee Surgery--a Comparison with the Dihydroergotamine-Heparin Combination. Ann Chir Gynaecol 85,4 (1996) : 359-63.

Roquebert, J., and B. Grenie. Alpha 2-Adrenergic Agonist and Alpha 1-Adrenergic Antagonist Activity of Ergotamine and Dihydroergotamine in Rats. Arch Int Pharmacodyn Ther 284,1 (1986) : 30-7.

Saper, J. R., and S. Silberstein. Pharmacology of Dihydroergotamine and Evidence for Efficacy and Safety in Migraine. Headache 46(2006) : S171-81.

Silberstein, S. D., and D. C. McCrory. Ergotamine and Dihydroergotamine: History, Pharmacology, and Efficacy. Headache 43,2 (2003) : 144-66.

Supornsilpchai, W., S. M. le Grand, and A. Srikiatkhachorn. Cortical Hyperexcitability and Mechanism of Medication-Overuse Headache. Cephalalgia 30,9 (2010) : 1101-9.

Supornsilpchai, W., S. M. le Grand, and A. Srikiatkhachorn. Involvement of Pro-Nociceptive 5-Ht2a Receptor in the Pathogenesis of Medication-Overuse Headache. Headache 50,2 (2010) : 185-97.

Supornsilpchai, W., S. Sanguanrangsirikul, S. Maneesri, and A. Srikiatkhachorn. Serotonin Depletion, Cortical Spreading Depression, and Trigeminal Nociception. Headache 46,1 (2006) : 34-9.

Tfelt-Hansen, P. C., and P. J. Koehler. History of the Use of Ergotamine and Dihydroergotamine in Migraine from 1906 and Onward. Cephalalgia 28,8 (2008) : 877-86.

Tozzi, A., A. de Iure, M. Di Filippo, C. Costa, S. Caproni, A. Pisani, P. Bonsi, B. Picconi, L. M. Cupini, S. Materazzi, P. Geppetti, P. Sarchielli, and P. Calabresi. Critical Role of Calcitonin Gene-Related Peptide Receptors in Cortical Spreading Depression. Proc Natl Acad Sci U S A 109,46 (2012) : 18985-90.

Villalon, C. M., D. Centurion, E. W. Willems, U. Arulmani, P. R. Saxena, and L. F. Valdivia. 5-Ht1b Receptors and Alpha 2a/2c-Adrenoceptors Mediate External Carotid Vasoconstriction to Dihydroergotamine. Eur J Pharmacol 484,2-3 (2004) : 287-90.

Villalon, C. M., J. Galicia-Carreon, A. Gonzalez-Hernandez, B. A. Marichal-Cancino, G. Manrique-Maldonado, and D. Centurion. Pharmacological Evidence That Spinal Alpha(2c)- and, to a Lesser Extent, Alpha(2a)-Adrenoceptors Inhibit Capsaicin-Induced Vasodilatation in the Canine External Carotid Circulation. Eur J Pharmacol 683,1-3 (2012) : 204-10.

Wang, X. M., Z. J. Zhang, R. Bains, and S. S. Mokha. Effect of Antisense Knock-Down of Alpha(2a)- and Alpha(2c)-Adrenoceptors on the Antinociceptive Action of Clonidine on Trigeminal Nociception in the Rat. Pain 98,1-2 (2002) : 27-35.

Wang, Y., R. Aun, and F. L. Tse. Brain Uptake of Dihydroergotamine after Intravenous and Nasal Administration in the Rat. Biopharm Drug Dispos 19,9 (1998) : 571-5.

Zhang, X., D. Levy, R. Noseda, V. Kainz, M. Jakubowski, and R. Burstein. Activation of Meningeal Nociceptors by Cortical Spreading Depression: Implications for Migraine with Aura. J Neurosci 30,26 (2010) : 8807-14.

Zhang, Z. G., M. Chopp, K. I. Maynard, and M. A. Moskowitz. Cerebral Blood Flow Changes During Cortical Spreading Depression Are Not Altered by Inhibition of Nitric Oxide Synthesis. J Cereb Blood Flow Metab 14,6 (1994) : 939-43.

APPENDICES

Appendix A

Western blot analysis solutions

1. 10x SDS-PAGE running buffer	1	liter
[250 mM Tris-HCl, 1.92 mM Glycine, 1% SDS)		
Tris-HCl (MW 121.14)	30.28	g
Glycine (MW 75.07)	144.13	g
Sodium dodecylsulfate (SDS)	10	g
Add dH_2O up to 1000 ml		
Store at room temperature		
2. 1x SDS-PAGE running buffer	1	liter
[25 mM Tris-HCl, 192 mM Glycine, 0.1% SDS]		
10x SDS-PAGE running buffer	100	ml
Add dH_2O up to 1000 ml		
Store at 4ºc		
3. 10x Transfer buffer	800	ml
[250 mM Tris-HCl, 1.92 mM Glycine]		
Tris-base (MW 121.14)	30.28	g
Glycine (MW 75.07)	144.13	g
Add ddH_2O 800 ml		
Store at room temperature		
4. 1x Transfer buffer	1	liter
10x Transfer buffer	80	ml
100% methanol	200	ml
Add dH_2O	720	ml
Store at 4ºc		

5. 10x Tris-buffer Saline (TBS)	1	liter
[100 mM Tris-HCl (pH 7.5), 150 mM NaCl]		
Tris-HCl (MW 121.14)	121.1	g
Add dH_2O almost ~ 900 ml and adjust pH to 7.5 by HCl		
NaCl (MW 58.4)	90	g
Add dH_2O up to 1000 ml		
Store at room temperature		

6. 10x TBS buffer	1	liter
[50 mM Tris-HCl (pH 7.6), 150 mM NaCl]		
Tris-HCl (MW 121.14)	60.57	g
NaCl (MW 58.4)	87.6	g
Add dH_2O almost ~800 ml and adjust pH to 7.6 by HCl		
Add dH_2O up to 1000 ml		
Store at room temperature		

7. 10% SDS (w/v)	100	ml
Weight out sodium dodecylsulfate (SDS)	10	g
Add dH_2O up to 100 ml		
Store at room temperature		

8. 4x Running Gel buffer	200	ml
[1.5 M Tris-HCl, pH 8.8]		
Tris-HCl (MW 121.14)	36.3	g
Add dH_2O almost ~ 150 ml and adjust pH to 8.8 by HCl		
Add dH_2O up to 200 ml		
Store at 4ºc		

9. 4x Stacking Gel buffer	50	ml
[0.5 M Tris-HCl, pH 6.8]		
Tris-HCl (MW 121.14)	3	g
Add dH_2O almost ~ 40 ml and adjust pH to 6.8 by HCl		
Add dH_2O up to 50 ml		
Store at 4ºc		
10. Coomassie blue staining	1	liter
[0.1% (w/v) Coomassie Brilliant Blue R250, 40% methanol, 10% glacial acetic acid]		
Coomassie Brilliant Blue R250	1	g
Methanol	400	ml
Stirr ~3 hr until dissolved, then add:		
Glacial acetic acid	100	ml
Add dH_2O up to 1000 ml * filter before store*		
Store at room temperature		
11. Destaining solution I	1	liter
[40 % methanol, 10 % acetic acid]		
Methanol	400	ml
Acetic acid	100	ml
Add dH_2O up to 1000 ml		
Store at room temperature		
12. Destaining solution II	1	liter
[10 % Methanol, 5 % Acetic acid]		
Methanol	100	ml
Acetic acid	50	ml
Add dH_2O up to 1000 ml		
Store at room temperature		

13. Ice-cold Tris buffer	500	ml
(5mM Tris-HCl, pH 7.4 autoclave)		
Tris- base (MW 121.14)	0.3	g
Add dH_2O almost ~ 400 ml and adjust pH to 7.4 by HCl		
Add dH_2O up to 500 ml		
Store at 4ºc		

14. RIPA buffer	100	ml
[50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM EDTA, 0.1% SDS, 1% Sodium deoxycholate, 1% Triton x-100]		
0.5 M Tris-HCl (MW 121.14)	10	ml
0.5 M NaCl (MW 58.4)	30	ml
0.5 M EDTA (FW 372.24)	2	ml
0.1 % SDS	0.1	g
1% Sodium deoxycholate	1	g
Triton x-100	1	ml
Store at 4ºc		

<u>0.5 M Tris-HCl pH 7.5</u>	50	ml
Tris-HCl	0.3	g
Add dH_2O almost ~ 80 ml and adjust pH to 7.5 by HCl		
Add dH_2O up to 50 ml		

<u>0.5 M NaCl</u>	100	ml
NaCl	2.92	g
Add dH_2O up to 100 ml		

15. 4X SDS Protein Sample Buffer (4X Loading dye)	10	ml
[240 mM Tris-HCl (pH 6.8), 40% Glycerol, 8% SDS, 0.04% Bromophenol blue, 5% β-mercaptoethanol]		
1 M Tris-HCl (pH 6.8)	2.4	ml
100 % Glycerol	4	ml
SDS	0.8	g
1% Bromophenol blue	0.4	ml
β-mercaptoethanol	0.5	ml
Add dH_2O	3.1	ml
Stable for week in the refrigerator or for months at -20ºc		
16. 1% Bromophenol blue (w/v)	10	ml
Bromophenol blue	0.1	g
Add dH_2O up to 10 ml		
Stir until dissolved		
*Filtration will remove aggregated dye		
Store at room temperature		
17. 5% Skim milk/BSA in TBST	25	ml
Skim milk powder/BSA	1.25	g
TBST	25	ml
Prepare freshly before use		
18. TBS with 0.1% Tween-20 (TBST)	1	liter
TBS	1000	ml
Tween-20	1	ml
Store at room temperature		

19. Stripping Membrane Solution	50	ml
14.3 M β-mercaptoethanol	0.35	ml
10% SDS	10	ml
Stacking 0.5 M Tris-Cl, pH 6.7	6.25	ml
Add dH_2O to 50 ml		

20. Developer	3.8	liter
Developer	415	g
Add dH_2O almost ~ 800 ml		
Boil at 56ºc		
Add dH_2O up to 3800 ml		

21. Fixer (1:4)	1	liter
Fixer	250	ml
Add dH_2O up to 1000 ml		

Appendix B

SDS-PAGE gel preparation

1. Separating gel solution for SDS-PAGE

(30% acrylamide stock, 37.5:1 acrylamide: bisacrylamide)

Running gel recipes for 1.5 and 0.75 mm thick gels (for 2 gels) Final gel concentration (10 ml; 2ea. 0.75-mm thick SE 250 gels)					
	5%	7.5%	10%	12.5%	15%
30% acrylamide	1.67 ml	2.5 ml	3.3 ml	4.2 ml	5 ml
4X running gel buffer (pH 8.8)	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml
10% SDS	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
ddH_2O	5.7 ml	4.9 ml	4.0 ml	3.2 ml	2.4 ml
Ammonium persulfate[1]	65 µl	65 µl	65 µl	65 µl	65 µl
TEMED[1]	5 µl	5 µl	5 µl	5 µl	5 µl

2. Stacking gel solution for SDS-PAGE

(30% Acrylamide stock, 37.5:1 acrylamide: bisacrylamide)

Stacking gel solutions (for 2 gels) Gel thickness: 0.75 mm. Total	3.361 ml
30% acrylamide	0.44 ml
4X stacking gel buffer (pH 6.8)	0.83 ml
10% SDS	33 µl
ddH_2O	2.03 ml
Ammonium persulfate[1]	25 µl
TEMED[1]	3 µl

[1] Added after deaeration

Appendix C

Western blot analysis protocol for c-Fos

1. Set up the gel cassette.

1.1 Using a pipette, transfer the 7.5% separating gel mixture to the gel cassette until it reaches a position 1 cm from the bottom of the sample loading comb.

1.2 To ensure that the gel sets with a smooth surface, very carefully run distilled water down one edge into the cassette using a pipette.

1.3 The gel can now be left to set (45-60 minutes). When set, a very clear refractive index change can be seen between the polymerized gels and overlaying water, pour off the overlaying water.

1.4 Add the stacking gel solution to the gel cassette until the solution reaches the cutaway edge of the gel plate.

1.5 Place the well-forming comb into this solution and leave to set. This will take about 30 min. refractive index changes around the comb indicate that the gel has set.

1.6 Carefully remove the comb from the stacking gel, assemble the cassette in the electrophoresis tank.

2. Electrophoresis

2.1 Fill the top reservoir with electrophoresis buffer ensuring that the buffer fully fills the sample loading wells, and looks for any leaks from the top tank.

2.2 Loading the sample

a. Boil 95°C, 10 minutes for sample.

b. Slowly deliver the sample and marker into the well. The dense sample solvent ensures that the sample settles to the bottom of the loading well.

2.3 The power pack is now connected to the apparatus. Ensure that the electrodes are arranged so that the proteins are running to the anode. Continue electrophoresis until the bromophenol blue reaches the bottom of the gel (100 volts for 90-120 minutes)

3. Blotting

3.1 Following electrophoresis, remove the gel from the gel apparatus, and place it on blotting filtrate paper that is submersed in transfer buffer. Immediately overlay the exposed side of the gel with the membrane (nitrocellulose or wetted PVDF). Overlay the PVDF membrane with another piece of blotting filter, and place the gel "sandwich" into the blotting chamber.

3.2 Connect the power pack electrodes to the blotting chamber (0.35 amperes for 90 minutes).

3.3 Disassemble the blotting chamber, and remove the membrane from the gel.

4. Staining

Staining total protein pattern on membrane with 0.2% (w/v) Ponceau S for 3-5 minutes.

5. Immunodetection

5.1. Incubation with antibodies

All steps are carried out with gentle agitation on a rocking agitator.

a. Block nonspecific binding sites with blocking solution (Bovine serum albumin or Non fat milk) overnight with 5% NFM for c-Fos and 1 hour with 5% NFM for β-actin.

b. Incubation 1 hour at room temperature with gentle agitation.

c. Incubation 3 hours in the primary antibody solution at 1:1,000 in 3% BSA/TBST for c-Fos. (Skip this step for β-actin)

d. Wash 10 minutes x 3 times in TBST

e. Incubate for 1-1.5 hours in the secondary antibody solution, anti-mouse at 1;10,000 in 5% BSA/TBST .

f. Wash 5 minutes x 3 times in TBST.

5.2. Enhanced Chemiluminescent detection

a. To detect the immunoreactive spots with ECL kit. Mix and immerse the membrane in ECL solution (about 5 minutes), ensuring that all surface of the membrane is covered with solution.

b. Place the membrane and cover it with a layer of wrap.

c. Superimpose the film on the membrane, expose the film for a time variable from 5 seconds to several minutes.

d. Develop the film with developer and fixer.

BIOGRAPHY

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