การเปลี่ยนแปลงการทำงานของไตเมื่อให้สารแอล-อาร์จีนินอย่างเฉียบพลันในสุนัข

นางสาวชุติมา อุดม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สหสาขาวิชาสรีรวิทยา
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
พ.ศ. 2538
ISBN 974-632-291-5

CHANGES IN RENAL FUNCTIONS DURING ACUTE L-ARGININE LOADING IN DOGS

MISS CHUTIMA UDOM

A Thesis Submitted in Partial Fulfillment of the Requirements
for the degree of Master of Science
Inter-Department of Physiology
Graduate School
Chulalongkorn University
1995

ISBN 974-632-291-5

Thesis Title	Changes in renal functions during acute L-arginine loading in dogs.
By	Miss Chutima Udom
Inter-Department	Physiology
Thesis Advisor	Professor Narongsak Chaiyabutr, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree.

Santi Thoongsuwan

.....Dean of Graduate School

(Associate Professor Santi Thoongsuwan, Ph.D.)

Thesis Committee

.....Chairman

(Associate Professor Bungorn Chomdej, Ph.D.)

.....Thesis Advisor

(Professor Narongsak Chaiyabutr, Ph.D.)

.....Member

(Associate Professor Ratree Sudsuang, Ph.D.)

.....Member

(Assistant Professor Choogiart Sucanthapree, Ph.D.)

ชุติมา อุดม : การเปลี่ยนแปลงการทำงานไตในสุนัขเมื่อให้สารแอล-อาร์จีนินอย่างเฉียบพลัน (CHANGES IN RENAL FUNCTIONS DURING ACUTE L-ARGININE LOADING IN DOGS) อ.ที่ปรึกษา : ศ.น.สพ.ดร.ณรงค์ศักดิ์ ชัยบุตร, 57 หน้า. ISBN 974-632-291-5

การวิจัยนี้มีจุดมุ่งหมายเพื่อศึกษา ผลของการให้สารแอล-อาร์จีนินอย่างเฉียบพลัน สัมพันธ์กับการสร้างสารไนตริกออกไซด์ ที่มีผลต่อการทำงานของไตและระบบไหลเวียนเลือดของสุนัข ว่ามีกลไกเกี่ยวข้องกับระบบเรนิน-แองจิโอเทนซินหรือพรอสทาแกลนดินหรือไม่ โดยให้สารแอล-เนม ซึ่งเป็นสารยับยั้งการสร้างสารไนตริกออกไซด์ (กลุ่ม 1), ให้สารแอล-เนมร่วมกับสารอินาลาพริล มาลีเอท (กลุ่ม 2) และให้สารแอล-เนมร่วมกับสารอินโดเมทธาซีน (กลุ่ม 3)

ผลการทดลองพบว่า การให้สารแอล-เนมอย่างเดียวมีผลต่อการทำงานของหัวใจและค่าความดันเลือดเฉลี่ยทั่วร่างกาย โดยอัตราการเต้นของหัวใจจะช้าลง ($P<0.05$) และค่าความดันเลือดเฉลี่ยทั่วร่างกายจะเพิ่มขึ้น ($P<0.001$) เมื่อเปรียบเทียบกับช่วงที่ให้สารแอล-อาร์จีนิน สารแอล-เนมยังมีผลต่อการทำงานของไต โดยพบว่า อัตราการไหลผ่านของพลาสม่าบริเวณไตและอัตราการไหลผ่านของเลือดบริเวณไตลดลง ($P<0.05$) และความต้านทานของหลอดเลือดบริเวณไตเพิ่มขึ้น ($P<0.01$) ส่วนความต้านทานรวมของหลอดเลือดส่วนปลายมีแนวโน้มเพิ่มขึ้น การให้สารแอล-เนมร่วมกับสารอินโดเมทธาซีน จะให้ผลไปในแนวเดียวกันกับกลุ่มที่ให้สารแอล-เนมอย่างเดียว แต่ผลต่อความต้านทานรวมของหลอดเลือดส่วนปลายจะเพิ่มขึ้นอย่างมีนัยสำคัญ ($P<0.001$) อัตราการขับปัสสาวะลดลงในทั้ง 2 กลุ่ม การขับทิ้งของโซเดียมมีแนวโน้มเพิ่มขึ้น ในสุนัขที่ให้สารแอล-เนมร่วมกับสารอินาลาพริล มาลีเอทพบว่า อัตราการเต้นของหัวใจลดลง ($P<0.01$) ค่าความดันเลือดเฉลี่ยทั่วร่างกายลดลงเล็กน้อย อัตราการไหลผ่านของพลาสม่าบริเวณไตและอัตราการไหลผ่านของเลือดบริเวณไตลดลงอย่างไม่มีนัยสำคัญ ความต้านทานของหลอดเลือดบริเวณไตเพิ่มขึ้นอย่างไม่มีนัยสำคัญ ส่วนอัตราการขับปัสสาวะลดลงเมื่อเทียบกับช่วงเวลาที่ให้สารแอล-อาร์จีนิน อัตราการขับทิ้งของโซเดียมลดลงเล็กน้อย ในการทดลองทั้ง 3 กลุ่ม ไม่พบการเปลี่ยนแปลงของระดับความเข้มข้นของโซเดียมในพลาสม่า แต่ในกลุ่ม 1 และกลุ่ม 3 ในช่วงที่ให้สารแอล-อาร์จีนินครั้งที่ 2 พบว่า ระดับความเข้มข้นของโพแทสเซียมและคลอไรด์ในพลาสม่าเพิ่มขึ้น ($P<0.01$) แต่ระดับความเข้มข้นของคลอไรด์ในพลาสม่าในกลุ่มที่ 3 ไม่เปลี่ยนแปลง

จากผลการทดลองนี้ชี้ให้เห็นว่าไนตริกออกไซด์สร้างจากสารแอล-อาร์จีนินได้ ซึ่งจะถูกยับยั้งได้โดยสารแอล-เนม ซึ่งการทำงานของไนตริกออกไซด์นั้น มีผลต่อการทำงานของไตและระบบไหลเวียนเลือดทั่วร่างกาย โดยกลไกการออกฤทธิ์ของไนตริกออกไซด์นั้น สัมพันธ์กับการทำงานของระบบเรนิน-แองจิโอเทนซิน แต่ไม่เกี่ยวข้องกับการทำงานของระบบพรอสทาแกลนดิน

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา สรีรวิทยา ลายมือชื่ออาจารย์ที่ปรึกษา .....

ปีการศึกษา 2537 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม —

C 445552 : MAJOR PHYSIOLOGY
KEY WORD : L-ARGININE / INDOMETHACIN / RENAL FUNCTIONS / DOGS / N^{ω}-NITRO-L-ARGININE METHYL ESTER
CHUTIMA UDOM : CHANGES IN RENAL FUNCTIONS DURING ACUTE L-ARGININE LOADING IN DOGS.
ADVISOR : PROF. NARONGSAK CHAIYABUTR, D.V.M.,Ph.D. 57 pp. ISBN 974-632-291-5

The aim of the present study was to study the effects of L-arginine which relating to nitric oxide synthesis on the renal function and general circulation in dogs. Whether these changes are mediated by renin-angiotensin or prostaglandin system during L-arginine loading following with administration of L-NAME; inhibitor of the nitric oxide synthesis (Group I) or following with administration of the combination of L-NAME and enalapril maleate (Group II) or following with administration of the combination of L-NAME and indomethacin (Group III).

The animals given L-arginine following with L-NAME alone, showed significantly decreased in heart rate ($P<0.05$) while mean arterial blood pressure was significantly increased ($P<0.001$)(Group I). In group I, a marked decrease in both renal plasma flow and renal blood flow ($P<0.05$) coincided with increase in renal vascular resistance ($P<0.01$) and total peripheral resistance was observed after L-NAME administration. By administration of the combination of L-NAME and indomethacin after L-arginine loading (Group III), the pattern of changes of general circulation and renal hemodynamics were similar way as those occured in group I. Total peripheral resistance was significantly increase ($P<0.001$) in this group. In both group I and groupII, urine flow rate were slightly decreased whereas urinary excretion of sodium was slightly increased. Administration of the combination of L-NAME and enalapril maleate after L-arginine loading (Group II), the decrease in heart rate ($P<0.01$), mean arterial blood pressure coincided with slightly increase in total peripheral resistance were noted. Renal plasma flow and renal blood flow were decreased in comparison with L-arginine infusion period. Urinary excretion of sodium was slightly decreased. In all groups, plasma concentation of sodium did not alter throughout the experimental period.In group I and II, plasma concentations of potassium and chloride in the fourth period were increased ($P<0.01$) but plasma concentation of chloride did not alter in group III.

These data may indicate that nitric oxide, which is synthesized from L-arginine, can be inhibited by L-NAME administration. The actions of nitric oxide on both renal hemodynamics and general circulation might possible relate to renin-angiotensin system but was not related to prostaglandin system.

ภาควิชา สัตวศาสตร์ สรีรวิทยา ลายมือชื่อนิสิต .....
สาขาวิชา สรีรวิทยา ลายมือชื่ออาจารย์ที่ปรึกษา .....
ปีการศึกษา 2537 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my advisors , Professor Dr.Narongsak Chaiyabutr for his kind advice , guidance , critical reading of manuscript and constant encouragement throughout this study.

It is a pleasure to acknowledge the kind assistance of Miss Siripen Komolvanich and Mr.Somchai Pondeenana in laboratory technique involvingchemistry. I am grateful , too ,to Miss Hathaitip Parkinsee for her help , sincerity and friendship. The others person who take care and look after the experimental animals.

I wish to extend my thanks to the Graduate School , Chulalongkorn University for funding supports and to the Department of Physiology , Faculty of Veterinary Science for providing of the facilities.

I am also indebted to all experimental dogs for their sacrifice which brought me to succeed in my study.

Finally , I am extremely grateful to my parent for their love , encouragement and everything given to me.

TABLE OF CONTENTS

Page

CHAPTER Page

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

ABBREVIATIONS

PCV	=	Packed cell volume	(%)
Hct	=	Hematocrit	(%)
HR	=	Heart rate	(beats/min)
CO	=	Cardiac output	(L/min)
SV	=	Stroke volume	(ml/min)
MAP	=	Mean arterial blood pressure	(mmHg)
TPR	=	Total peripheral resistance	(mmHg/L/min.)
GFR	=	Glomerular filtration rate	(ml/min/kg.bw.)
RPF	=	Renal plasma flow	(ml/min/kg.bw.)
RBF	=	Renal blood flow	(ml/min/kg.bw.)
RVR	=	Renal vascular resistance	(mmHg/min/ml.)
FE_{Na}	=	Fractional excretion of sodium	(%)
FE_K	=	Fractional excretion of potassium	(%)
FE_{Cl}	=	Fractional excretion of chloride	(%)
FF	=	Filtration fraction	(%)
V	=	Urine flow rate	(µl/min.)
P_e	=	Plasma concentration of electrolytes	(mEq/L)
U_eV	=	Urinary excretion of electrolytes	(mM/min)
kg.bw.	=	kilogram of body weight	
mg.	=	milligram	

mmHg.	=	millimeter mercury
min.	=	minute
L-ARG	=	L-arginine
L-NAME	=	N^{ω}-nitro L-arginine methyl ester
NO	=	Nitric Oxide
EDRF	=	Endothelium-derived relaxing factor
IND.	=	indomethacin
ENA.	=	Enalapril maleate
Na	=	Sodium
K	=	Potassium
Cl	=	Chloride
PAH	=	Para-amino-hippurate
P_{in}	=	Plasma concentration of inulin
P_{PAH}	=	Plasma concentration of PAH
U_{in}	=	Urinary concentration of inulin
U_{PAH}	=	Urinary concentration of PAH

CHAPTER I

INTRODUCTION AND AIMS

It is well recognized that amino acid loading can cause an acute rise in glomerular filtration rate (GFR) and renal plasma flow (RPF) in animals (Johannessen, Lie and Kul, 1977 ; Woods, Mizelle and Hall, 1987 ; Woods et al., 1986) and humans (Castellino et al., 1988 ; Castellino et al., 1987). Mechanisms operated through hormones such as glucagon (Hirschberg et al., 1988), growth hormone, prolactin, insulin and insulin like growth factor I, (Guler et al., 1989; Hirschberg et al., 1988 ; Visek., 1985), macula densa and tubuloglomerular feedback (Woods et al., 1987 ; Appianni et al., 1988) have been proposed. L-arginine is the most interesting of all amino acid because it can be synthesized by the renal cortex (Hirschberg et al., 1988). The deficiency of L-arginine can reduce glomerular filtration rate, renal plasma flow and urine flow rate but filtration fraction rise, its mechanism remains unclear. Effects of acute L-arginine loading on renal and systemic hemodynamics in dogs have indicated that renal and systemic vasodilatation were in a dose-dependent despite indomethacin (IND) effect, and the direct effect of L-arginine on contractility of the heart. The effect of L-arginine has been indicated to affect both systemic and renal hemodynamics, but the effect on renal hemodynamics is independent from systemic circulation (Napathorn et al., 1992).

It has been reported that L-arginine is the physiological precursor for nitric oxide (NO) synthesis by endothelial cells which is endothelium-derived relaxing factor (Furchgott, 1983 ; Ignarro, 1989) or it calls that nitric oxide-endothelium derived relaxing factor (NO-EDRF). Nitric oxide endothelium derived relaxing factor can induce vasodilatation which enhancing glomerular filtration rate and reduce renal vascular resistance (Radermacher,

Forstermann and Frolich., 1990). The most of nitric oxide is synthesized in renal cortex (Walder, Thiemermann and Vane, 1991). The cGMP (cyclic guanidino monophosphate) is the mediator of EDRF (Burton et al., 1990). Effects of L-arginine loading can reduce renal vascular resistance and increase in glomerular filtration rate by mediated mesangial cells (Deng and Baylis, 1993). Nitric oxide may be mediated by acetylcholine and bradykinin (Hoffend et al., 1993). Nitric oxide has been inhibited by competitive L-arginine or L-arginine derivative such as L-NAME (N^{ω}-Nitro-L-arginine methyl ester). The experiment of L-NAME activity in vivo could not reveal the technique for mesurement of nitric oxide synthesis, but the experiment in vitro has been indicated that L-NAME is a specific inhibitor of nitric oxide endothelium-derived relaxing factor by competitive with the precusor of nitric oxide synthesis (Rees et al., 1989 ; Radermacher et al., 1991).

The lowest dose of L-NAME has been shown to decrease the rate of urine flow with no alteration in either sodium excretion or renal hemodynamics (Lahera et al., 1991). The medium dose of L-NAME can reduce the fall in excretion of sodium with the decrease in urine flow rate. The fall in filtration fraction and antinatriuresis has been shown to occur without changes in mean arterial pressure (MAP), renal plasma flow (RPF) or glomerular filtration rate (GFR). At a high dose of L-NAME, a marked increase in glomerular resistance with a fall in glomerular filtration rate and the filtration fraction have been reported. The intrarenal mechanism responsible for these effects remains unclear. Recent evidence suggests that EDRF is NO or a related nitroso compound and it is enzymatically generated from the basic amino acid, L-arginine. The renal vascular resistance increased occuring before changes in systemic blood pressure. The renal vascular bed has been shown more sensitive than other vascular territories, but with increasing doses of L-NAME, these begining to vasoconstrict, resulting in an elevated blood pressure. Especially, in rat has been shown to affect to feedback mechanism of decreased sodium reabsorption in the proximal tubules and increased sodium excretion, these alterations have not been mediated by renin-

angiotensin systems (Baylis et al., 1993). However, this mechanism has not been reported in the dogs. Little is known about endothelium-dependent vasodilatation in renal resistance vessels and the mechanism for such effects on salt remains unclear.

The purpose of the present study was to investigate the role of L-arginine relating nitric oxide on renal function and general circulation in vivo in dog. Whether its physiological roles are mediated via renin-angiotensin systems or prostaglandin systems using concomitant acute blockade of L-NAME, inhibitor of the nitric oxide synthesis.

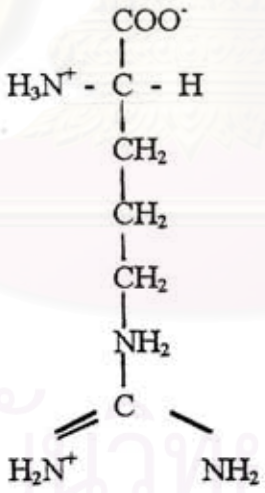
CHAPTER II

BACKGROUND INFORMATION

L-arginine.

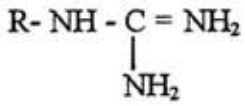
L-arginine is the essential of the common amino acids some paper calls semiessensial amino acid because it can synthesis in the kidney and liver in adult animals and human. L-arginine is a precursor of nitric oxide by endothelium derived relaxing factor.

The structure of L-arginine follow:



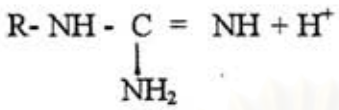
(L-arginine)

Acid form of L-arginine:



(guanidinium)

Base form of L-arginine:



(guanidino)

The terminal guanidino nitrogens of L-arginine are the physiological precursor of endothelium-derived NO (Harald et al., 1988). L-arginine is the source of nitric oxide (NO) which is produced by vascular endothelium as the muscle relaxing agent.

The kidney is the major biosynthetic source of circulating arginine in the rat was first reported by Featherston et al., (1973) and the availability of citrulline is a limiting factor for renal arginine synthesis in rats. In their studies with rats , injected (^{14}C) citrulline was incorporated into muscle protein as (^{14}C) arginine but only in animals with functional kidneys. The kidney readily synthesizes arginine from the citrulline, and this citrulline can arise from the intestinal metabolism of glutamine (Windmueller et al., 1981), the existence of the intestinal renal axis that converts glutamine to arginine. The microtechnique shown that arginine synthesis in the proximal tubule, with a progressively decreasing intensity from it early part (convoluted tubule adjacent to the glomerulus) to it more distal part (medullary pars recta). Very little L-arginine formation was found in other nephron segments(Levillian et al., 1990). Arginine is synthesized from citrulline by the third and fourth enzymes of the urea cycle, argininosuccinate synthase and argininosuccinate lyase. For many years it was assumed that the citrulline and arginine found in the circulation of the rat were produced by the urea cycle in liver.

Effect of L-arginine on the renal hemodynamics.

The previous studies shown that arginine depletion cause profound and reversible changes in the isolated perfused kidney of the rat : renal perfusion flow rate (PFR), glomerular filtration rate (GFR), and urine flow rate (UFR) decreased , and filtration fraction (FF) increased (Radermacher et al., 1991). Function dependent on filtered load are altered in relation to changes in GFR. There is no obvious direct tubular effect, as functions mainly located in the distal tubule and collecting duct (like potassium excretion and reabsorption of free water) are not influenced by arginine depletion or repletion. Because hemodynamic parameters were altered, it is difficult to simultaneously evaluate changes in tubular function. In 1992 Napathorn et al, studied in dogs on the different dose of L-arginine (low-dose and high dose) on the renal hemodynamics and systemic hemodynamics. On renal hemodynamics, GFR and RPF were slightly increased whereas RVR was slightly decreased during the low dose of L-arginine so that the weak renal vasodilatation effect of L-arginine in the presence of prostaglandins in normal conditions. When prostaglandin synthesis is blocked , the predominant action of vasoconstrictive hormone leads to a reduction in RPF without an alteration in GFR. When infusion of high dose of L-arginine after IND-treated, GRF and RPF rised and RVR reduced. It seems that L-arginine infusion stimulates the release of other vasodilators. Growth hormone and glucagon are among the possibilities.

Effect of L-arginine on systemic hemodynamics.

The low dose of L-arginine and IND administration ; increased total peripheral resistance (TPR), mean arterial blood pressure (MAP) was slightly increased. An

increased in vascular resistance thus reduces the venous return to the heart but cardiac output (CO) little changed. The high dose of L-arginine decreased TPR and increased CO whereas a low dose did not. L-arginine induced systemic vasodilatation and increased CO in a dose-dependent manner. When infused the second times of high dose of L-arginine after IND treatmented TPR and MAP little changed but increased CO. These are shown that the direct effect of L-arginine on contractility of the heart (Napathorn et al., 1992).

Endothelium- dependent relaxing factor and Nitric oxide (NO).

Furchgott (Furchgott and Vanhoutte, 1989) recently found that many familiar vasodilatation substances, such as acetylcholine, histamine, bradykinine and other peptides, produce this effect only in vessels with an intact endothelium. With lengths of artery suspended in an organ both, the relaxant effect is abolished if the endothelium is removed simply by gently rubbing the inside of the vessel. Such a denuded vessel will, however, still relax in response to the perfusate from an endothelium-intact vessel exposed to one of these relaxant agents. The unknown mediator was termed endothelium-derived relaxing factor (EDRF).

This simple and important discovery stimulated a scientific gold-rush by many groups to be the first to identify the unknown mediator, which was generally assumed to be either a peptide or an eicosanoid an easy pushover, it was supposed. Attempts to identify it were, however, initially frustrated by its very short lifetime of only a few seconds in plasma, and it was not until 1987 that the surprising answer was obtained that the unknown mediator is nitric oxide (NO) (Moncada et al, 1989). The first clues came from studies showing many similarities between EDRF and nitrate vasodilators; in particular, the effects of both are associated with increased cGMP formation in smooth

muscle cells and inhibition by methylene blue, an inhibitor of guanylate cyclase. It was known that nitrates act by being converted to NO, so this became a likely candidate for EDRF. The matter was settled by Moncada and Palmer, who showed that isolated endothelial cells do in fact generate NO when exposed to many vasodilator mediators. Subsequent work has shown that the source of NO is the amino acid arginine, and that the enzyme responsible for it production, NO synthase, is activated by a rise in calcium intracellular in the endothelium cells. These cells have reports for various mediators, many of which act via inosital phosphate formation to raise calcium intracellular, thus accounting for the large number of agents that exert this effect.

NO causes smooth muscle relaxation by activating a cytosolic from of guanylate cyclase, thus increasing cGMP formation though various cGMP-dependent protein kinases are known, and we can be confident that protein phosphorylation is the mechanism by which control is exerted, the details of how increased cGMP cause relaxation are not yet clear (Ignarro and Kadowitz, 1985).

There is now increasing evidence that NO acts as a mediator in other situation also, for example in macrophages and in the central nervous system.

Nitric oxide (NO)

The demonstration in 1987 of the formation of NO by an enzyme in vascular endothelial cells opened up what can now by considered a new area of biological research (Moncada et al, 1989). NO, which accounts for the biological properties of endothelium derived relaxing factor (EDRF), is the endogenous stimulator of the soluble guanylate cyclase.

Early observations of endothelium-dependent relaxation and endothelium-derived relaxation factor from 1980 to 1987.

In 1980, Furchgott and Zawadzki demonstrated that the vascular relaxation induced by acetylcholine (Ach) was dependent on the presence of the endothelium and provided evidence that this effect was mediated by a labile humoral factor, later known as EDRF.

The discovery of the formation of NO L-arginine by mammalian tissues and the elucidation of some of its biological roles has, in the last 4 years, thrown new light onto many areas of research. NO is released under physiological conditions by a constitutive, Ca^{2+}-dependent enzyme in response to receptor stimulation. This L-arginine : NO pathway is the transduction mechanism whereby cells regulate their own function or communicate with others.

In the cardiovascular system the release of NO acts as a general adaptive mechanism whereby the vascular endothelium responds to changes in its environment and regulates blood flow and blood pressure through an action on the vascular smooth muscle. In addition, NO regulates the interaction between the endothelium and the platelets and probably blood - borne cells, and it may also play a role in the control of vascular smooth muscle proliferation.

The generation of NO also as an autocrine regulatory system, for platelets do not seem to transfer NO to other platelets or cells but modulate their own ability to aggregate by generating NO. At present there are no other examples of this autocrine function. However, the increases in cyclic GMP that follow receptor stimulation in same cells are likely to depend on NO generated by those same cells.

The importance of the L-arginine : NO pathway in the nervous system has yet to be established fully, although it is already known to be linked to the stimulation by the excitatory amino acids of specific receptors in the central nervous system. Because both the NO synthase and the soluble guanylate cyclase are widely and not uniformly distributed in the brain, it is likely that this pathway is associated with other mediator

system, not only in the central nervous system but also in both the sensory and motor areas of the peripheral system. Furthermore, the presence of the NO synthase has been demonstrated in the adrenal medullar and the retina, where it may be involved in regulation of cathecholamine release and in the gaiting mechanism for light-sensitive neurons, respectively.

The NO released by the constitutive enzyme may also play regulatory roles in other cells. So far, this enzyme has been found in the adrenal cortex and some epithelial cell lines, suggesting that it may participate in the regulation of the secretion or action of other hormones.

The way in which L-arginine is made available to the NO pathway and the relative importance of this metabolic route in relation to others for L-arginine metabolism remains to be elucidated. It seems that in vivo and in vitro, unless cells are maintained in L-arginine-free media, there is sufficient intracellular L-arginine to supply the Ca^{2+}-dependent NO synthase. However, when cells are stimulated in vitro to generate NO, it is then possible to supply exogenous L-arginine to this enzyme. Indeed, this seems to be the case in vascular endothelial cells, platelets, and neutrophils. Endothelial cells that have been depleted of L-arginine are able to synthesize this amino acid from endogenous sources (Hecker et al., 1990; Sessa et al., 1990) suggesting that under normal circumstances the availability of L-arginine is well regulated. A different situation may operate for the inducible, Ca^{2+}-independent enzyme because, once induced, this enzyme releases NO for long periods, and in macrophages in vitro, for example, it leads to a depletion of L-arginine in the medium or to their own death. A decrease in the levels of L-arginine, either locally or systematically, may be involved in hypertension, vasospasm, or atherosclerosis.

Role of the renin angiotensin system in regulating renal hemodynamic.

Angiotensin II is a potent renal vasoconstrictor. When exogenous angiotensin II is administered into the kidney there is an increase in both afferent and efferent arteriole resistance. It has been reported that an infusion of low doses of angiotensin into the circulation appears to be associated with sodium retention whereas high dose infusion tend to be associated with increases in sodium and water excretion. Under certain condition (particularly in sodium retaining states), angiotensin II appears to be one of the regulators of renal vascular resistance, since the administration of the angiotensin II antagonist, saralasin is associated with an increase in renal blood flow. The study on the action of angiotensin by microperfusion techniques in proximal tubule in the rat has shown that low dose administration of angiotensin II in the peritubular perfusion fluid stimulated sodium reabsorption whereas inhibition of sodium reabsorption was seen at much higher dose.

There is the evidence that angiotensin may have a direct effect on the glomerulus. Administration of angiotensin II has been shown to alter the dynamics of glomerular filtration. In addition to altering glomerular capillary pressure, angiotensin II has been shown to decrease the ultrafiltration coefficient of the glomerulus. Some studies have suggested that there are specific binding sites for angiotensin II in glomerulus and that these binding sites may be associated with the surface of mesengial cells. The effect of angiotensin II on the glomerulus appears to be blocked by the administration of saralasin. There was evidence that angiotensin II generated locally within the kidney can influence renal function under conditions of sodium and water restriction. Angiotensin may play an important role in regulation the rate of aldosterone secretion. One of the most important factors in the control of aldosterone secretary rate is the level of activity of the renin-angiotensin system. In addition to angiotensin II,

potassium and sodium concentrations all play important roles in regulating the rate of aldosterone secretion. Administration of angiotensin II into blood vessels supplying the brain results in an increase in peripheral blood pressure. An increase in blood pressure induced by angiotensin administered centrally has been reported in dog and rabbit, these central effects are mediated mainly by increased efferent sympathetic activity. There is an interaction between angiotensin II and the sympathetic nervous system in facilitation of adrenergic neurotransmission. So it appears that the facilitation of sympathetic neurotransmission by angiotensin II is due to an increase in norepinephrin in the synaptic cleft.

Prostaglandins

Prostaglandins are derivatives of twenty-carbon, monocarboxylic acids

In mammalian cells there are two major pathways of arachiolonic acid metabolism that produce important mediators of cellular and bodily function : the cyclooxygenase and the lipoxygenase pathways. The substrate for both pathways is unesterified arachidonic acid. The cycloxygenase pathway leads to a series of compound including prostaglandins and thromboxanes. The prostaglandins were discovered through their effects on smooth muscle, specifically their ability to promote the contraction of intestinal and uterine muscle and the lowering of blood pressure. Although the complexity of their structures and the diversity of their sometimes conflicting function often create a sense of frustration, the potent pharmacological effects of the prostaglandins have afforded them an important place in human biology and medicine. With the exception of the red blood cell, the prostaglandins are produced and released by nearly all mammalian cells and tissues ; they are not confined to specialized cells as insulin is to the pancreas. Furthermore, unlike most other hormones,

the prostaglandins are not stored in cells but instead are synthesized and released immediately.

There are three major classes of primary prostaglandins, the A, E and F series. They are all related to prostanoic acid (Figure A).

Figure A. Structure of prostanoic acid

Synthesis of prostaglandins involves a cyclooxygenase

The immediate precursors to the prostaglandins are C_{20} polyunsaturated fatty acids containing 3,4 and 5 carbon-carbon double bonds. Since arachidonic acid and most of its metabolites contain 20 carbon atoms, they are referred to as eicosanoids. During their transformation into various prostaglandins they are cyclized and take up oxygen. Dikomo-γ-linoleic acid (C_{20}-48,11,14) is the precursor to PGE_1 and $PGF_{1\alpha}$; arachidonic acid (C_{20}-45,8,11,14) is the precursor to PGE_2 and $PGE_{2\alpha}$; and eicosopentaenoic acid (C_{20}-45,8,11,14,17) is the precursor to PGE_3 and $PGE_{3\alpha}$ (Figure B).

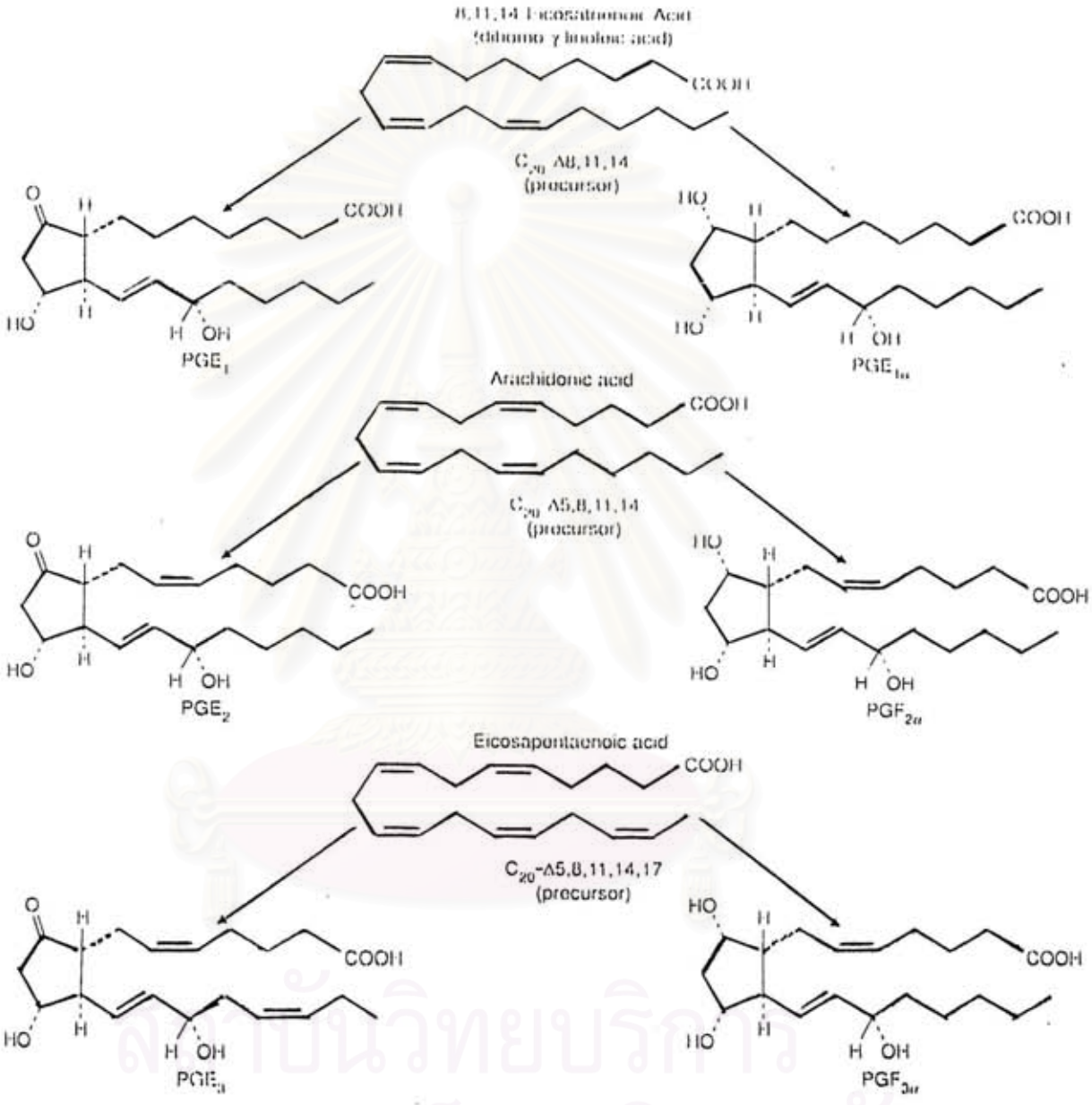


Figure B. **Synthesis of E and F prostaglandins from fatty acid precursors.**

Compounds of the 2-series derived from arachidonic acid are the principal prostaglandins in humans and are of the greatest significance biologically. Thus, one should focus attention primarily on the metabolism of arachidonic acid (Figure C).

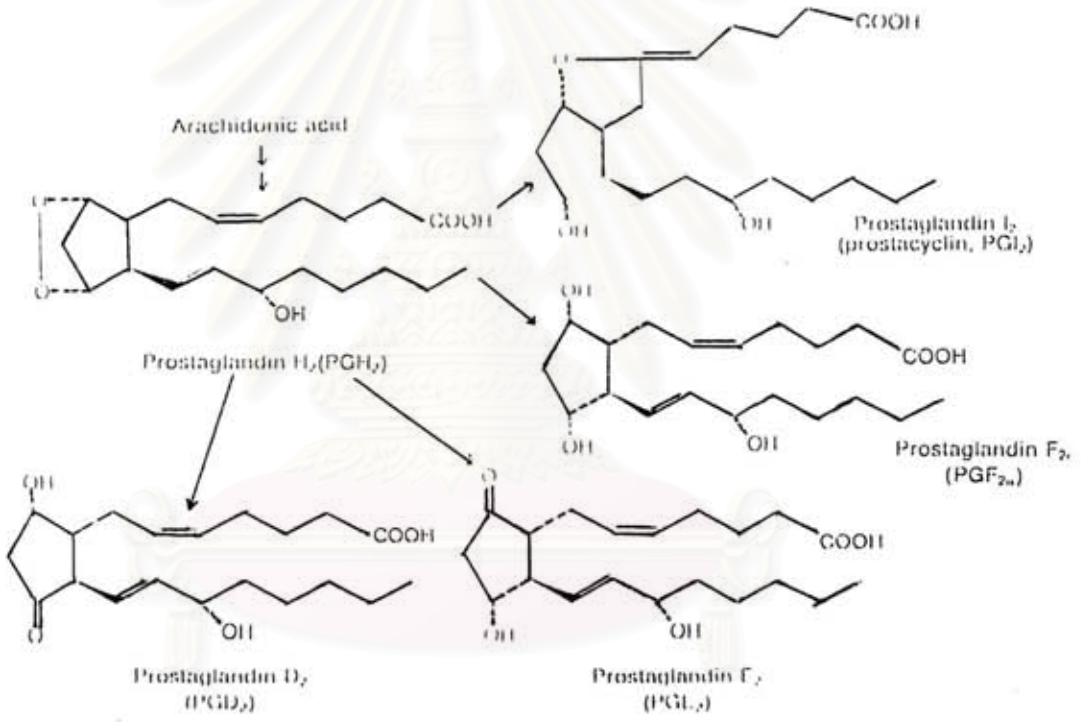
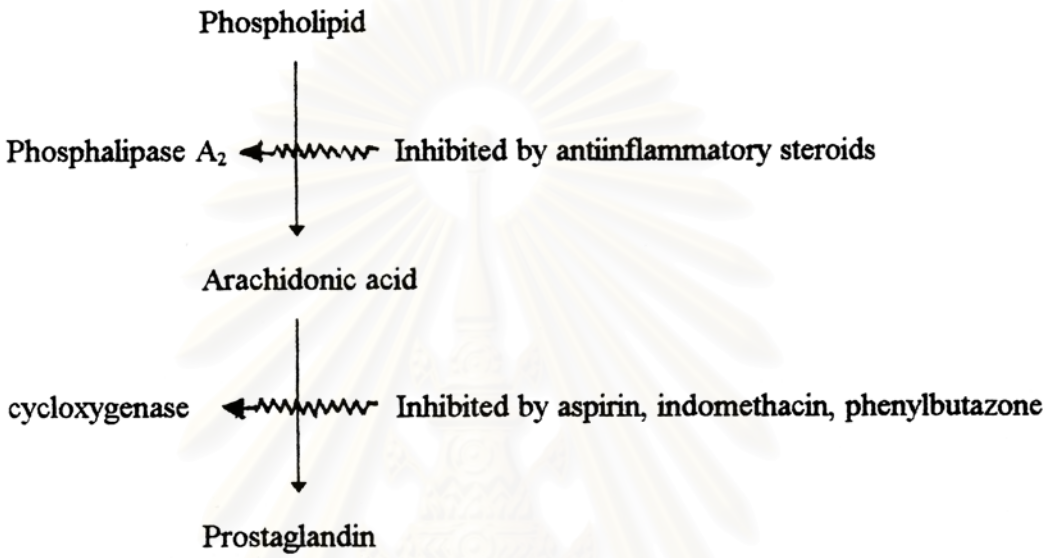


FIGURE C.
Major routes of prostaglandin biosynthesis.

The prostaglandins have a very short half-life. Soon after release they are rapidly taken up cells and inactivated. The lungs appear to play an important role in inactivating prostaglandins.

Site of action of inhibitors of prostaglandin synthesis



Regulation of blood pressure : Prostaglandins play an important role in controlling blood vessel tone and arterial pressure. The vasodilator prostaglandins, PGE, PGA, and PGI_2, lower systemic arterial pressure, thereby increasing local blood flow and decreasing peripheral resistance. Thromboxanes A_2 causes contraction of vascular smooth muscle and glomerular mesangium. There is hope that the prostaglandins may eventually prove useful in the treatment of hypertension.

CHAPTER III

MATERIALS AND METHODS

Animal preparations

The experiments were preformed on 15 adult male mongrel dogs, weighing between 9-18 kgs. The dogs were devided into three groups, 5 dogs in each group. The animals were fasted for 12 hours prior to the investigation. On the day of experiment, the dog was anasthetized with pentobarbital sodium 30 mg/kg.bw. intravenously and were subsequently given small maintenance dose as necessary. A tracheal canula was inserted to secure free airway. Two femoral veins were canulated with polyethylene tube (PE 180). One for infusion of solution containing inulin and para-aminohippurate (PAH) the other one for infusion of 0.9% saline and L-arginine solution. Before the clearance study; the animal was given supplemental 0.9% saline 10 ml/kg.bw. and the priming solution containing PAH 1.2 gm% and inulin 5 gm% in isotonic saline solution. It was administered intravenously 0.5 ml/kg.bw.and followed immediately by sustaining solution composed of 0.12 gm% and 0.5 gm% of PAH and inulin respectively at the rate of 1.5 ml/min. The rate of infusion was kept constant throughout the period of experiment by a peristaltic pump (Eyla Model 3). One of femoral artery was cannulated with polyethylene tube (PE 200) for blood collection and connected to the pressure transducer (PE 23AA) for recording blood pressure and heart rate by polygraph (Grass model 7).

The left ureter was reached by lateral flank incisions with a retroperitoneal approach and tubulated with polyvinyl catheter (PV 190) for urine collection. After an hour of infusion of inulin and PAH solution, and the rate of urine flow stabilized, urine samples were obtained. The blood sample was collected at the midpoint of the urine collection. As shown in Figure D.

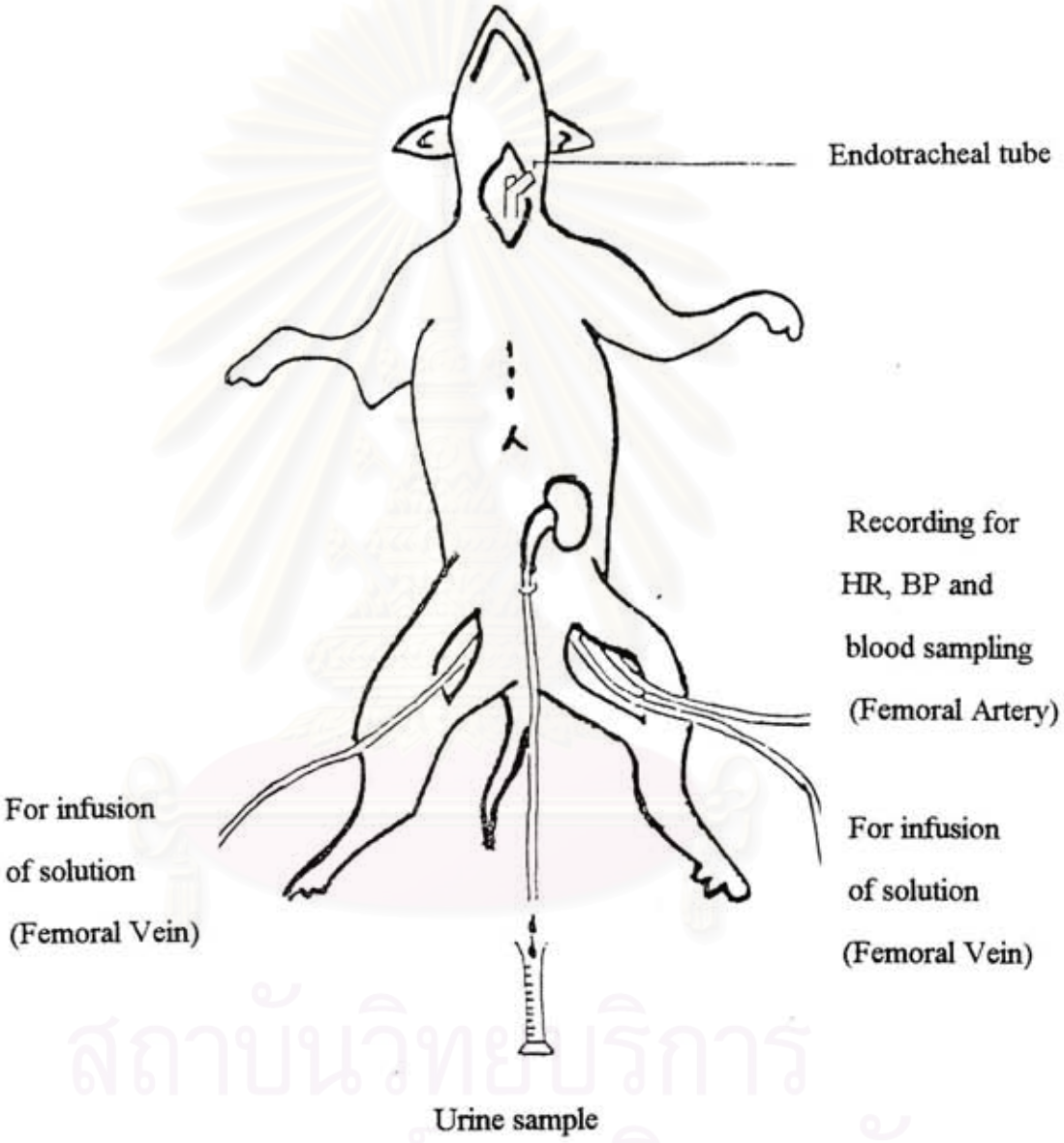


Figure D. Scheme of experiment

Experimental procedures

Three groups and five animals each were used to study the effects of L-arginine loading on the renal function and general circulation. In each group, experiment was divided for four experimental periods; the control period with normal saline infusion; the pretreatmented period with L-arginine infusion; the treatmented period of L-NAME in group I, L-NAME combination with enalapril maleate in group II and L-NAME combination with indomethacin in group III and the posttreatment with the same L-Arginine loading. Experiments were carried out as following:

Group I.

Effect of L-arginine loading following to L-NAME administration on renal function and general circulation.

Five dogs were used to study in four periods. Prior to experiment, a minimum about 60 minutes was allowed for equilibration and stabilization. The first period (the control period) the animal was infused intravenously by 1.5 ml/min of normal saline for 30 minutes. The second period (the pretreatment period) was infusion by L-arginine 2.5 mmol/kg.bw. as the same rate of the control period for 30 minutes. The third period (the treatment period) was injected by a bolus dose of L-NAME 50 mg/kg.bw. and infused normal saline continue for 30 minutes. The fourth period (the posttreatment period) was infused by L-arginine the same dose of 2.5 mmol/kg.bw. for 30 minutes. After 30 minutes in each period, the blood sample was collected at the midpoint of the urine collection. The systemic blood pressure and heart rate were recorded.

Group II.

Effects of L-arginine loading following the combination of L-NAME and enalapril maleate administration on renal function and general circulation.

Five dogs were treated in the same manner as in group I but in the treatment period, animal was injected intravenously the by bolus dose of L-NAME 50 mg/kg.bw. with enalapril maleate (AII. blocker) 10 mg/kg.bw.

Group III.

Effects of L-arginine loading following the combination of L-NAME and indomethacin administration on renal function and general circulation.

Five dogs were treated in the same manner as in group I but in the treatment period; animal was injected intravenously the by bolus dose of L-NAME 50 mg/kg.bw. with indomethacin 10 mg/kg.bw.

Experimental protocols :

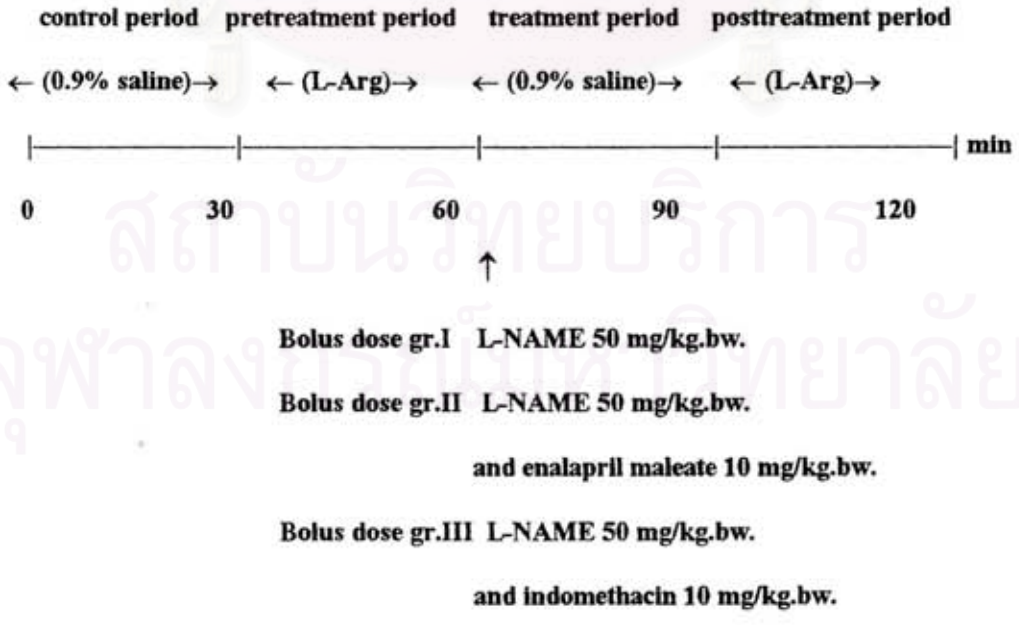


Figure E. Diagrammatic illustration of the experimental protocols.

Determination of cardiac output

Cardiac output (CO) was measured by dye dilution technique, using Evans blue (T-1824). Cardiac output was measured by using technique as described by Chaiyabutr et al.,(1980) 1.5 ml of a bolus dose of T-1824 (0.5%) was injected into femoral vein. Then series of blood sameple were collected from the femeral artery immediately after dye injection. Serial samples of arterial blood were collected by means of peristaltic pump and fraction collection. Each of sample approximately 1 ml/sec. was collected for a period of 10-14 second. Then the amount of dye in each blood sample was determined respectively by spectophotometry. Cardiac output was calculated as described by Chaiyabutr et al.,(1980). Packed cell volume was determined by the preparation of blood in an international microcapillary centrifuge and measured with Hawksley microhematocrit reader.

Determination of plasma and urine samples

Plasma and urine inulin concentrations were determines by the anthrone method as described by Young and Raiss (1952). Determination of plasma and urine PAH concentration were carried out by the method of Bratton and Marshall as modified by Smith (1962).

The sodium and potassium concentration in plasma and urine were determined by flame photometer (Corning; clinical Flame Photometer 410C) chloride by chloridometer (Corning; chloride Analyzer 925)

Calculation:

Glomerular filtration rate (GFR) = $\frac{UinV}{Pin}$

Renal plasma flow (RPF) = $\frac{U_{PAH}V}{P_{PAH}}$

Renal blood flow (RBF) = $\frac{RPF \times 100}{(100\text{-}PCV)}$

Filtration fraction (FF) = $\frac{GFR \times 100}{RPF}$

Fractional electrolytes excretion = $\frac{UeV/Pe \times 100}{GFR}$

Mean arterial blood pressure (MAP) = $\frac{Ps+2Pd}{3}$

Total peripheral resistance (TPR) = $\frac{MAP}{CO}$

Renal vascular resistance (RVR) = $\frac{MAP}{RBF}$

Urinary electrolyte excretion = UeV

$$\text{Urine flow rate} = \frac{\text{Urine volume}}{\text{time(min)}}$$

$$\text{Stroke volume (SV)} = \frac{\text{CO}}{\text{HR}}$$

Statistical analysis

All data presentration were normalized to individual body weight to allow comparison among the dogs. Data were reported as the mean value ± S.E. The paired t-test was used to estimate the statistical significance of differences between value obtained from the control period and from each period of the experiment.

CHAPTER IV

RESULTS

Effects of L-arginine loading following L-NAME administration on general circulation.

Effects of L-NAME administration following L-NAME administration on the general circulation (group I) are shown in table I. By L-arginine loading alone did not affect to values of hematocrit (Hct), stroke volume (SV), cardiac output (CO), mean arterial blood pressure (MAP) and total peripheral resistance (TPR). Heart rate (HR) decreased significantly from the control period from 138±11 to 126±9 beats/min. ($P<0.05$). By given L-arginine loading following with L-NAME administration, HR still be significantly decreased from the control period (from 138±10 to 114±8 beats/min, $P<0.01$) which was by approximately 17% (Fig.1). MAP was significantly increased (from 114.7±6.6 to 136.3±7.9 mmHg, $P<0.01$) and it was significantly increased (from 110.0±6.4 to 136.3±7.9 mmHg, $P<0.001$) in comparison with the control value, which was by approximately 24% (Fig.2). The CO and SV were slightly decreased while TPR was slightly increased. The Hct value did not show any changes during L-arginine loading following with L-NAME administration. However, when L-arginine was given after L-NAME administration the significant decrease in Hct and HR were noted, which were 15% ($P<0.05$) and 21% ($P<0.01$) respectively. MAP still be increased insignificantly.

The changes of plasma sodium did not alter throughout the experimental period. Ingroup I and II, plasma concentrations of potassium and chloride in the fourth period were increased ($P<0.01$) but plasma concentration of chloride did not alter in group III.

Effects of L-arginine loading following L-NAME administration on renal function.

Effects of L-arginine loading following with L-NAME administration on the renal function in group I are shown in table II. By giving L-arginine loading alone did not affect to glomerular filtration rate (GFR), renal plasma flow (RPF), renal blood flow (RBF) and renal vascular resistance (RVR). When L-NAME was given after L-arginine loading the significant decrease in RPF and RBF have been observed (from 7.26±1.16 to 4.17±0.39 and 9.05±1.41 to 5.22±0.49 ml/min/kg.bw, $P<0.05$ respectively). The RPF was decreased from 7.88±0.95 to 4.17±0.39 ml/min/kg.bw ($P<0.01$), which was by approximately 46% (Fig. 6) and the RBF was decreased from 10.06±1.26 to 5.22±0.49 ml/min.kg.bw ($P<0.01$) by approximately 47% (Fig. 6) in comparison with the control period. RVR was increased significantly (from 13.77±2.07 to 27.32±3.59 mmHg/min/ml, $P<0.01$) and from the control value (from 11.70±1.83 to 27.32±3.59 mmHg/min/ml, $P<0.01$), which was by approximately 140% (Fig.7). GFR was slightly decreased when compared to those either in L-arginine loading period alone or the control period (Fig.7). In the period L-arginine loading after L-NAME administration, both RPF and RBF still be decreased (4.17±0.39 vs 3.45±0.62 ml/min/kg.bw), which were approximately 56% and 58% respectively ($P<0.01$) when compare to control period (Fig.8). RVR still be increased (27.32±3.59 vs 37.85±7.15 mmHg/min/ml) and it was significantly increased (from 11.70±1.83 to 37.85±7.15 mmHg/min/ml, $P<0.05$) from the control value. During L-arginine alone, urine flow rate was significantly increased from the control period (from 30.41±0.19 to 86.07±0.31 µl/ min.kg.bw, $P<0.01$). L-NAME administration after L-arginine loading caused a significant increased in the urine flow rate from 30.41±0.19 to 74.10±0.31µl/min/kg.bw, $P<0.01$) in comparison with the control value, however, this value was not significantly different from the urine flow rate during L-arginine loading alone. By giving L-arginine loading after L-NAME administration, urine flow rate still be significant increased

($P<0.01$) when compared to the control period. The marked increment in urine flow rate when compared to these in L-NAME administration (74.10±0.31 vs 102.29±0.33 µl/min/ kg.bw, $P<0.05$) has been noted.

Effects of L-NAME administration after L-arginine on electrolyte excretion in animals (group I) are shown in table III. Fractional excretion of sodium (FE_{Na}) and chloride (FE_{Cl}) did not showed any significant changes when given L-arginine alone but not fractional excretion of potassium (FE_K). Urinary electrolyte excretion for sodium ($U_{Na}V$) was slightly increased from 0.63±0.23 to 1.65±0.63 µmol/min (Fig.8) and for chloride ($U_{Cl}V$) showed marked increase ($P<0.001$) from 0.03±0.05 to 3.89±0.40 µmol/ min. When L-arginine given alone. Urinary excretion for potassium (U_KV) (Fig.8) did not show any change. When L-NAME was given after L-arginine loading, FE_{Na} still increased in comparison with control value from 0.4±0.2 to 1.2±0.4% but it decreased when compared to L-arginine loading period from 2.3±1.5 to 1.2±0.4%. The data for FE_K and FE_{Cl} were slightly increased from the control and FE_K was increased significantly (13.8±2.3 vs 18.5±2.4 %) ($p<0.01$) in comparison with L-arginine loading alone but FE_{Cl} was slightly increased. $U_{Na}V$ and U_KV slightly increased from the control and from L-arginine loading (Fig. 8). $U_{Cl}V$ was increased significantly ($P<0.05$) from the control and L-arginine loading (Fig. 9). L-arginine loading follow L-NAME administration. FE_{Na}, FE_K and FE_{Cl} slightly increased from the control and in comparison with L-NAME administration. $U_{Na}V$ and U_KV increased insignificantly from the control (Fig. 8). $U_{Cl}V$ increased significantly ($P<0.05$) from the control 0.03±0.05 to 7.06±1.88 µmol/min (Fig. 9). When L-arginine loading alone, filtration fraction (FF) was increased significantly ($P<0.05$) from the control (Fig. 7). When L-NAME administration following L-arginine loading, FF increased significantly ($P<0.01$) from the control from 20.48±2.13 to 36.07±3.99% and incomparison with L-arginine loading the value from 25.41±3.21 to 36.07± 3.99%. L-arginine loading after L-NAME administration FF also increased significantly ($P<0.05$) from the control 20.48 ± 2.13 to 43.89 ± 6.91% (Table II).

Effects of L-arginine loading following with combination of L-NAME and enalapril maleate administration on general circulation.

Effects of L-arginine loading following with combination of L-NAME and enalapril maleate administration on general circulation (group II) are shown in table I. No changes of Hct, HR, SV, CO, MAP and TPR were seen when L-arginine infusion alone. L-NAME combination with enalapril maleate was given after L-arginine loading, a marked decrease in HR was noted while Hct, SV, CO, MAP and TPR were not affected. The animals were given L-arginine loading after the combination of L-NAME and enalapril maleate, it was found that Hct was slightly decreased and it was decreased significantly ($P<0.05$) compared to the control from 29.4±1.8 to 24.7±2.6%, HR was slightly decreased and it was decreased significantly (from 158±11 to 122±15 beats/min, $P<0.05$) from the control value (Fig.1). MAP was slightly increased (Fig. 2). There were no changes in MAP value during L-arginine loading alone and during L-NAME combination with enalapril maleate administration.

Effects of L-arginine loading following with combination of L-NAME and enalapril maleate administration on renal function.

Effects of L-arginine loading following with combination of L-NAME and enalapril maleate administration on renal function (group II) are shown in table II. L-arginine loading alone was not affect to GFR, RPF, RBF and RVR. The combination of L-NAME and enalapril maleate was administered after L-arginine loading, GFR showed no changed while RPF decreased significantly (from 5.78±0.86 to 3.37±0.41 ml/min/kg.bw, $P<0.05$) from in comparison with L-arginine loading alone, which was by approximately 59% (Fig.5) from the control value. The RBF was slightly decreased with slightly increased in RVR from the control value (Fig. 7) but the RBF was decreased significantly from L-arginine loading (from 8.30±1.47 to 4.75±0.63 ml/min/kg.bw, $P<0.05$) (Fig. 6) while the RVR was increased significantly from

L-arginine loading (from 14.60±2.40 to 23.96±3.16 ml/min/kg.bw, $P<0.05$). When the animals were given L-arginine loading again, GFR slightly decreased coincided with slgiht decreases in RPF and RBF. RVR increased nearly 33%. During L-arginine loading alone the urine flow rate was increased significantly from the control (from 43.62±0.09 to 79.93±0.35 µl/min/kg.bw, $P<0.05$) (Fig. 5). When L-NAME combination with enalapril maleate was given to the animals, the urine flow rate was still higher in comparison with the control period but this rate was slightly low in comparison to L-arginine alone (Table II).

Effects of L-arginine loading following with combination of L-NAME and enalapril maleate administration on electrolyte excretion animals (group II) are shown in table III. L-arginine loading alone did not affect to FE_{Na} and FE_K. The FE_{Na} was slightly decreased, the FE_K was slightly increased but the FE_{Cl} marked increased significantly ($P<0.05$). These changes coincided with $U_{Na}V$, U_KV and $U_{Cl}V$ (Fig 8,.9). The FF was slightly decreased from the control (Table II, Fig. 7). When L-NAME combination with enalapril maleate administration following L-arginine loading alone, the FE_{Na} and FE_K were slightly decreased while FE_{Cl} was decreased significantly (from 5.4±1.2 to 2.7±0.8 %, $P<0.01$). The $U_{Na}V$ was decreased and U_KV was increased insignificantly. But $U_{Cl}V$ did not any altered from the control value and slightly decreased from pre-L-arginine loading. When L-arginine loading after combination of L-NAME and enalapril maleate administration, FE_{Na}, FE_K and FE_{Cl} were slightly incresesd. FE_{Na} and FE_K were slightly increased while FE_{Cl} was increased significantly ($P<0.05$) from the control value. $U_{Na}V$ and $U_{Cl}V$ were slightly increased but not U_KV. The $U_{Na}V$ was slightly decreased, the U_KV did not any alterated but $U_{Cl}V$ was increased significantly ($P<0.05$) from the control value. The FF was increased insignificantly (Table II, Fig.7).

Effects of L-arginine loading following with combination of L-NAME and indomethacin administration on general circulation

Effects of L-arginine loading following with combination of L-NAME and indomethacin administration on general circulation in group III are shown in table I. L-arginine loading alone did not affect to value of SV. The Hct was decreased significantly ($P<0.05$) from the control value. HR, CO, MAP and TPF were slightly decreased from the control value. The animals were given combination of L-NAME and indomethacin administration following L-arginine loading alone. The Hct was slightly decreased. HR showed marked decreased significantly (from 129 ± 8 to 104 ± 8 beats/min, $P<0.01$) and CO was decreased significantly (from 1.95 ± 0.27 to 1.15 ± 0.26 l/ min, $P<0.05$). In comparison with the control period, Hct was slightly decreased (Fig. 1). HR showed marked decreased significantly ($P<0.01$) from the control value (Fig.1) but CO was slightly decreased and SV did not any alterations (Table I). MAP has been shown increased significantly ($P<0.001$) from the control value and compared to L-arginine loading period. The TPR was increased significantly from the control value and compared to L-arginine loading period ($P<0.01$ and $P<0.05$), respectively). L-arginine loading after combination of L-NAME and indomethacin administration, The Hct and HR were slightly decreased but not MAP. The Hct was decreased significantly ($P<0.05$) from the control which was by approximately 17% (Fig.1). The HR showed marked decrease significantly ($P<0.001$), which was by approximately 17% (Fig.1) and the MAP marked increase significantly ($P<0.001$) from the control value, which were by approximately 60% (Fig.2).

Effects of L-arginine loading following with combination of L-NAME and indomethacin administration on renal function.

Effects of L-arginine loading following with combination of L-NAME and indomethacin administration on renal function in group III are shown in table II.

L-arginine loading alone the GFR, RPF and RBF were slightly increased. The RVR did not alteration from the control. When L-NAME combination with indomethacin was given following L-arginine loading GFR was decreased significantly (from 1.17±0.1 to 0.66±0.08 ml/min/kg.bw, $P<0.05$) and it was slightly decreased from the control, which was by approximately 30% (Fig.7). RPF and RBF were decreased significantly ($P<0.01$) and they were decreased significantly from the control value ($P<0.01$), which were by approximately 60% (Fig.6). The RVR was increased significantly 15.70±2.04 to 82.02±26.07 mmHg/min/ml, $P<0.05$) and it was increased signification ($P<0.05$) from the control value which was by approximately 400% (Fig.7). When L-arginine loading after combination of L-NAME and indomethacin administration GFR was slightly decreased but it was decreased significantly ($P<0.05$) from the control value, which was by 18% (Fig.5). RPF and RBF were slightly decreased and they were significantly ($P<0.01$ and $P<0.001$, respectively) from the control value. The RVR was increased insignificantly ($P<0.01$) but it was increased significantly but it ($P<0.01$) from the control value, which was by approximately 700% (Fig.7). When L-arginine loading alone the urine flow rate was increased significantly ($P<0.01$) from the control value. L-NAME combination with indomethacin was given to animals the urine flow rate slightly was decreased from L-arginine loading alone. When L-arginine loading after combination of L-NAME and indomethacin administration, the urine flow rate increased has been shown insignificantly from the control value from 79.22±0.26 to 94.72±0.63 µl/min/kg.bw.

Effects of L-arginine loading following with combination of L-NAME and indomethacin administration on electrolyte excretion in animal group III are shown in table III. When L-arginine loading alone FE_{Na} was slightly increased. FE_K has been shown slightly decreased. FE_{Cl} was increased significantly from the control (from 1.7±0.8 to 4.9±1.8 %, $P<0.05$). $U_{Na}V$, U_KV and $U_{Cl}V$ were increased insignificantly (Fig.8,9). The FF did not any alteration (Table II). The animals were given L-NAME combination with indomethacin. FE_{Na}, FE_K and FE_{Cl} were increased insignificantly and they were slightly increased from the control value but FE_{Cl} was increased

significantly ($P<0.05$). $U_{Na}V$ and $U_{Cl}V$ were increased insignificantly. U_KV did not any altered from the control. FF showed marked increased significantly ($P<0.01$) and it was increased significantly from the control value (from 23.16±2.24 to 38.98±3.92 %, $P<0.01$) (Table II). When L-arginine loading after combination of L-NAME and indomethacin administration FE_{Na}, FE_K and FE_{Cl} were slightly increased insignificantly and they have been noted increased insignificantly from the control value. $U_{Na}V$ was slightly decreased but it was slightly increased from the control value. U_KV was decreased insignificantly from the control value and comparison with combination of L-NAME and indomethacin administration. $U_{CL}V$ has been shown increased insignificantly from the control and it did not any alteration from combination of L-NAME and indomethacin administration. The FF showed marked increase significantly ($P<0.01$) and little changed in comparison with combination of L-NAME and indomethacin administration (Table II).

Table I. Changes in general circulation in dogs response to intravenous infusion of L-NAME alone (Group I) , L-NAME and enalapril maleate (Group II.) and L-NAME and indomethacin (Group III.) by compared with L-arginine infusion in all groups.

	Hct (%)	HR (beats/min)	MAP (mmHg)	SV (ml/min)	CO (L/min)	TPR (mmHg/min/L)
GROUP I.						
CONTROL	21.3±1.4	138±11	110.0±6.4	30.85±4.83	4.59±1.09	25.86±6.36
L-ARG	19.9±0.9	126±9*	114.7±6.6	30.78±6.06	4.14±0.72	29.12±5.37
L-NAME	20.0±1.2	114±8** $^{+}$	136.3±7.9*** $^{++}$	28.28±8.71	3.44±0.93	50.70±17.75
L-ARG	18.0±1.3*	109±8**	141.7±9.7**			
GROUP II						
CONTROL	29.4±1.8	158±11	111.7±7.5	14.71±3.16	2.37±0.62	59.29±13.29
L-ARG	28.7±2.9	171±16	109.0±9.4	16.34±3.93	2.61±0.38	44.85±20.06
L-NAME+ENA	28.4±3.1	134±12** $^{+}$	107.3±7.2	12.61±1.98	1.62±0.21	69.60±09.17
L-ARG	24.7±2.6*	122±15*	121.0±13.7			
GROUP III						
CONTROL	34.8±2.7	131±6	107.2±10.2	13.98±2.40	1.99±0.32	67.34±12.51
L-ARG	30.8±2.6*	129±8	108.3±10.7	14.45±1.94	1.95±0.27	64.91±7.32
L-NAME+IND	31.8±3.1	104±8** $^{++}$	166.9±11.2*** $^{+++}$	14.34±1.81	1.15±0.26^{+}	126.94±13.29*** $^{++}$
L-ARG	29.1±2.7*	105±8***	171.1±13.0***			

Significant difference values using paired t-test are indicated by *P<0.05, **P<0.01,***P<0.001 compared to control value of each group.
Significant difference values using paired t-test are indicated by $^{+}$P<0.05, $^{++}$P<0.01,$^{+++}$P<0.001 compared to L-arginine loading.
Mean±S.E.

Table II. Changes in renal function in dogs response to intravenous infusion of L-NAME alone (Group I) , L-NAME and enalapril maleate (Group II.)and L-NAME and indomethacin (Group III.) by compared with L-arginine infusion in all groups.

	GFR (ml/min/kg.bw.)	RPF (ml/min/kg.bw.)	RBF (ml/min/kg.bw.)	RVR (mmHg/min/ml)	FF (%)	V (μl/min/kg.bw.)
GROUP I						
CONTROL	1.54±0.11	7.88±0.95	10.06±1.26	11.70±1.83	20.48±2.13	30.41±0.19
L-ARG	1.73±0.17	7.26±1.16	9.05±1.41	13.77±2.07	25.41±3.21*	86.07±0.31**
L-NAME	1.51±0.25	4.17±0.39** $^{+}$	5.22±0.49** $^{+}$	27.32±3.59** $^{++}$	36.07±3.99** $^{++}$	74.10±0.31**
L-ARG	1.43±0.23	3.45±0.62**	4.20±0.74**	37.85±7.15*	43.89±6.91*	102.29±0.33*
GROUP II						
CONTROL	0.94±0.18	4.77±0.83	6.68±1.07	18.46±3.08	20.71±3.18	43.62±0.09
L-ARG	0.87±0.09	5.78±0.86	8.30±1.47	14.60±2.40	17.65±4.61	79.93±0.35*
L-NAME+ENA	0.94±0.19	3.37±0.41 $^{+}$	4.75±0.63 $^{+}$	23.96±3.16 $^{+}$	29.04±4.98 $^{+}$	61.03±0.23
L-ARG	0.69±0.15	3.18±0.67	4.33±1.08	35.62±9.70	26.10±7.69	85.42±0.42
GROUP III						
CONTROL	1.03±0.11	4.58±0.60	6.94±0.68	16.02±1.91	23.16±2.24	47.67±0.23
L-ARG	1.17±0.12	5.02±0.66	7.15±0.68	15.79±2.04	24.31±2.98	107.90±0.49**
L-NAME+IND	0.66±0.08^{+}	1.83±0.31** $^{++}$	2.74±0.49** $^{++}$	82.02±26.07* $^{+}$	38.98±3.92** $^{++}$	79.22±0.26
L-ARG	0.50±0.10*	1.21±0.26**	1.75±0.40***	121.64±21.92**	41.40±4.27**	94.72±0.63

Significant difference values using paired t-test are indicated by *P<0.05, **P<0.01,***P<0.001 compared to control value of each group.
Significant difference values using paired t-test are indicated by $^{+}$P<0.05, $^{++}$P<0.01,$^{+++}$P<0.001 compared to L-arginine loading.
Mean±S.E. Used the only left kidney.

Table III. Changes in urinary electrolyte excretion and fractional excretion (FE) in dogs response to intravenous infusion of L-NAME alone (Group I) L-NAME and enalapril maleate (Group II.) and L-NAME and indomethacin (Group III.) by compared with L-arginine infusion in all dogs.

	$U_{Na}V$ (μM/min/kg.bw.)	FE_{Na} (%)	U_KV (μM/min/kg.bw.)	FE_K (%)	$U_{Cl}V$ (μM/min/kg.bw.)	FE_{Cl} (%)
GROUP I						
CONTROL	0.63±0.23	0.4±0.2	0.83±0.10	17.0±1.7	0.33±0.05	1.9±1.7
L-ARG	1.65±0.63	2.3±1.5	0.83±0.16	13.8±2.3*	3.89±0.40***	2.3±0.2
L-NAME	2.33±0.79	1.2±0.4	1.01±0.17	18.5±2.4^{++}	3.86±0.88*$^{+}$	2.5±0.4
L-ARG	4.27±1.57	2.5±1.1	1.19±0.25	21.5±4.0	7.06±1.88*	5.1±1.5
GROUP II						
CONTROL	3.01±1.12	2.1±0.6	1.09±0.33	34.4±8.1	2.28±0.89	2.1±0.7
L-ARG	2.18±0.85	1.8±0.7	1.21±0.40	46.5±14.7	4.64±0.96*	5.4±1.2*
L-NAME+ENA	1.69±0.70	1.2±0.4	1.21±0.29	37.5±10.9	2.48±0.56	2.7±0.8^{++}
L-ARG	2.44±1.28	2.2±0.8	1.08±0.30	44.5±13.4	4.94±0.99*	7.7±1.8*
GROUP III						
CONTROL	2.64±1.21	1.7±0.6	1.03±0.21	31.1±4.3	2.09±1.08	1.7±0.8
L-ARG	3.41±1.69	2.2±1.1	1.15±0.43	26.4±10.2	6.17±1.84	4.9±1.8*
L-NAME+IND	5.29±2.45	5.1±2.1	1.01±0.20	37.9±5.9	5.19±2.12	8.1±2.4*
L-ARG	8.14±5.48	9.1±5.8	0.85±0.41	40.1±17.4	8.96±5.04	14.2±6.6

Significant difference values using paired t-test are indicated by *P<0.05, **P<0.01,***P<0.001 compared to control value of each group.
Significant difference values using paired t-test are indicated by $^{+}$P<0.05, $^{++}$P<0.01,$^{+++}$P<0.001 compared to L-arginine loading.
Mean±S.E. Used the only left kidney.

Table IV Changes in plasma concentration of electrolytes in dogs response to intravenous infusion of L-NAME alone (Group I) , L-NAME and enalapril maleate (Group II.) and L-NAME and indomethacin (Group III.) by compared with L-arginine infusion in all groups.

	P_{Na} (mEq/L)	P_K (mEq/L)	P_{Cl} (mEq/L)
GROUP I			
CONTROL	133.2±4.7	3.2±0.2	99.6±1.3
L-ARG	135.6±1.6	3.4±0.1	102.6±1.4
L-NAME	135.6±1.5	3.7±0.2	101.8±3.0
L-ARG	134.6±1.4	3.9±0.2	103.0±0.8
GROUP II			
CONTROL	136.8±1.1	3.2±0.2	103.2±1.6
L-ARG	136.0±1.1	3.4±0.2	103.0±1.3
L-NAME+ENA	140.2±1.7	3.6±0.3	104.4±1.4
L-ARG	138.4±1.4	3.8±0.3	102.2±1.2
GROUP III			
CONTROL	131.2±2.4	3.2±0.1	101.2±0.8
L-ARG	131.7±1.3	4.6±1.4	101.8±1.7
L-NAME+IND	136.0±3.2	3.9±0.2	102.3±1.1
L-ARG	136.0±1.3	4.0±0.2	102.2±0.5

Significant difference values using paired t-test are indicated by *P<0.05, **P<0.01,***P<0.001 compared to control value of each group.
Significant difference values using paired t-test are indicated by $^{+}$P<0.05, $^{++}$P<0.01,$^{+++}$P<0.001 compared to L-arpinine loading.
Mean±S.E.

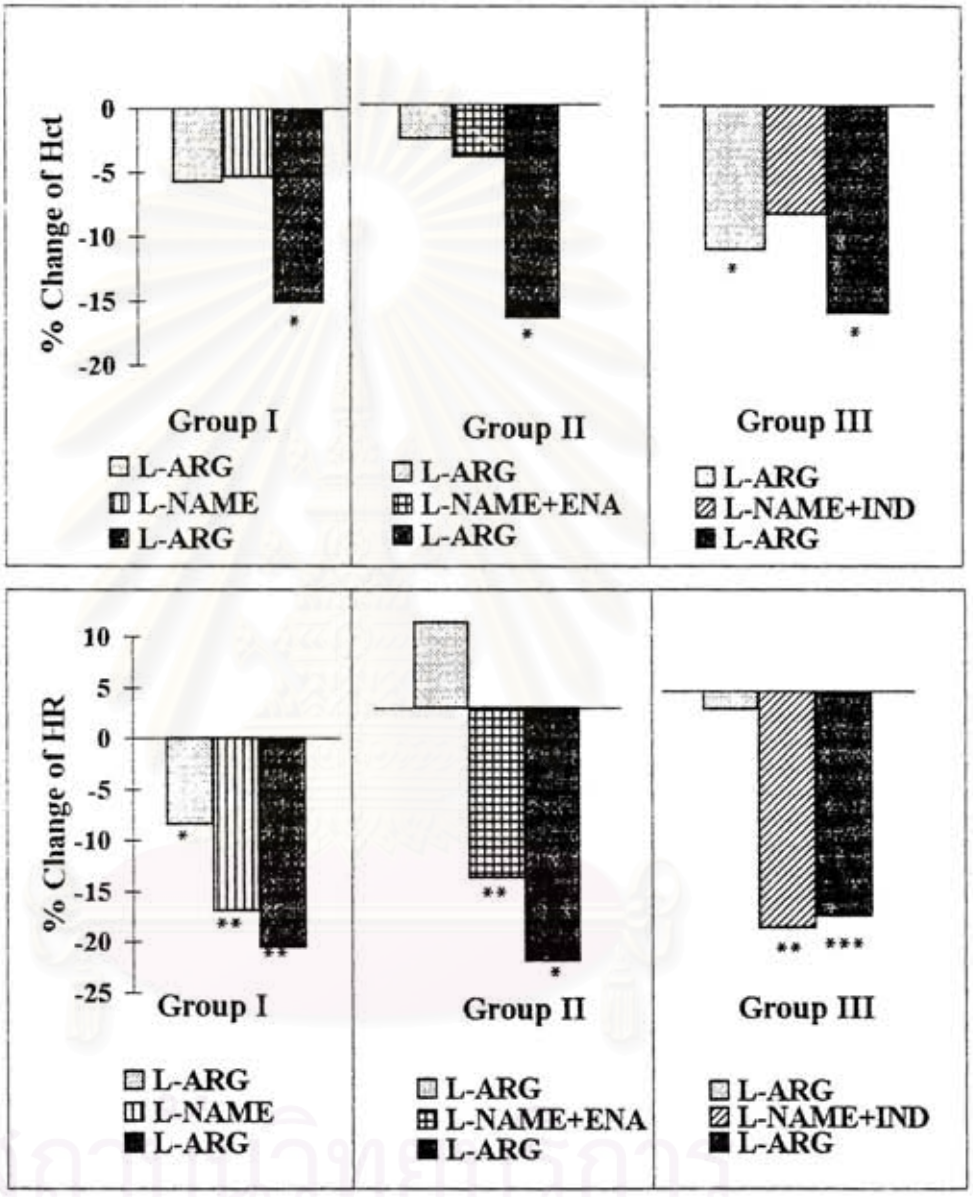


Figure 1 Percentage changes in hematocrit (Hct) and heart rate (HR) in animals given L-arginine loading during administration of L-NAME alone (Group I); administration of combination of L-NAME and enalapril maleate(ENA) (Group II) and administration of combination of L-NAME and indomethacin (IND) (Group III).
The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

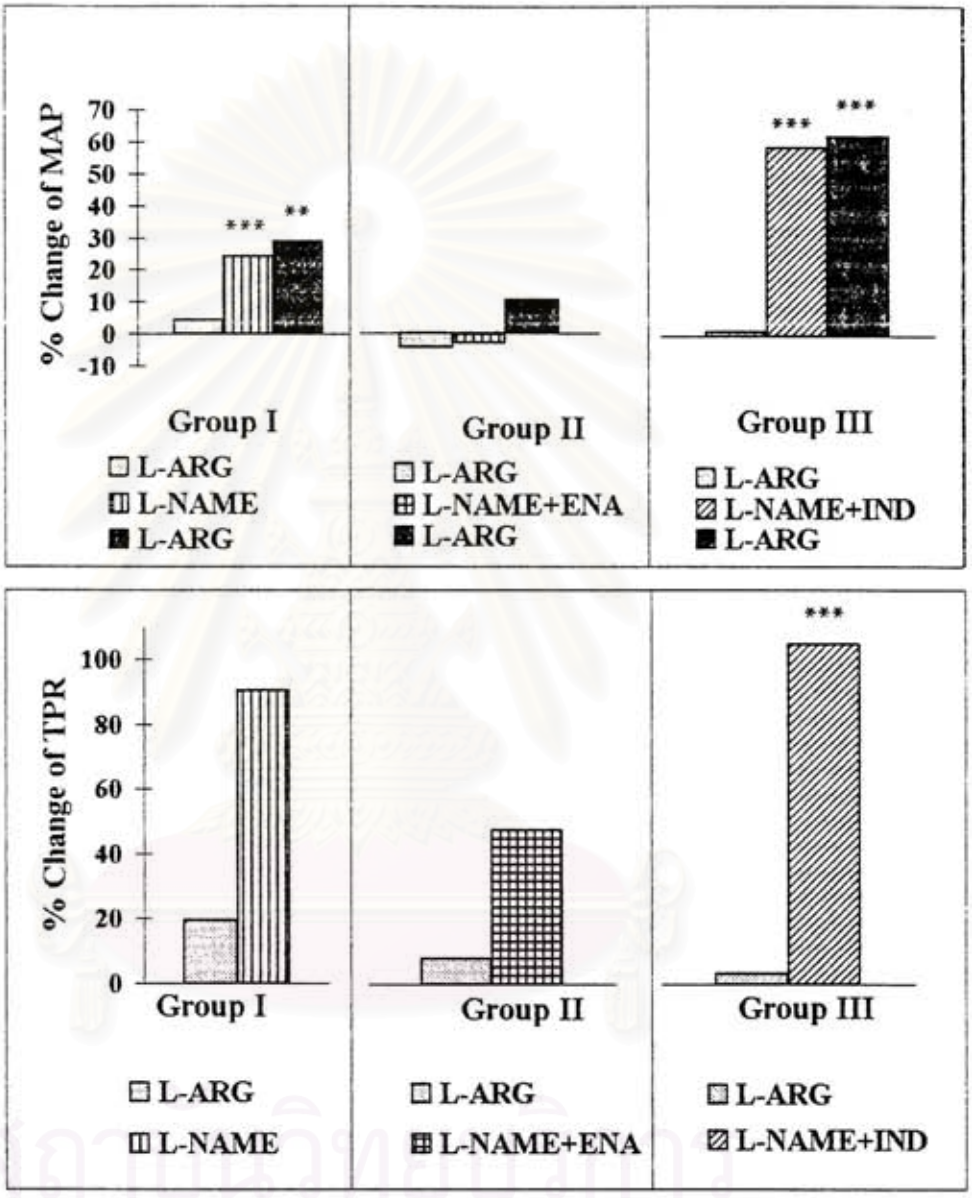


Figure 2 Percentage changes in mean arterial blood pressure (MAP) and total peripheral resistance (TPR) in animals given L-arginine loading during admnistration of L-NAME alone (Group I) ; administration of combination of L-NAME and enalapril maleate (ENA) (Group II) and administration of combination of L-NAME and indomethacin (IND) (Group III).
The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

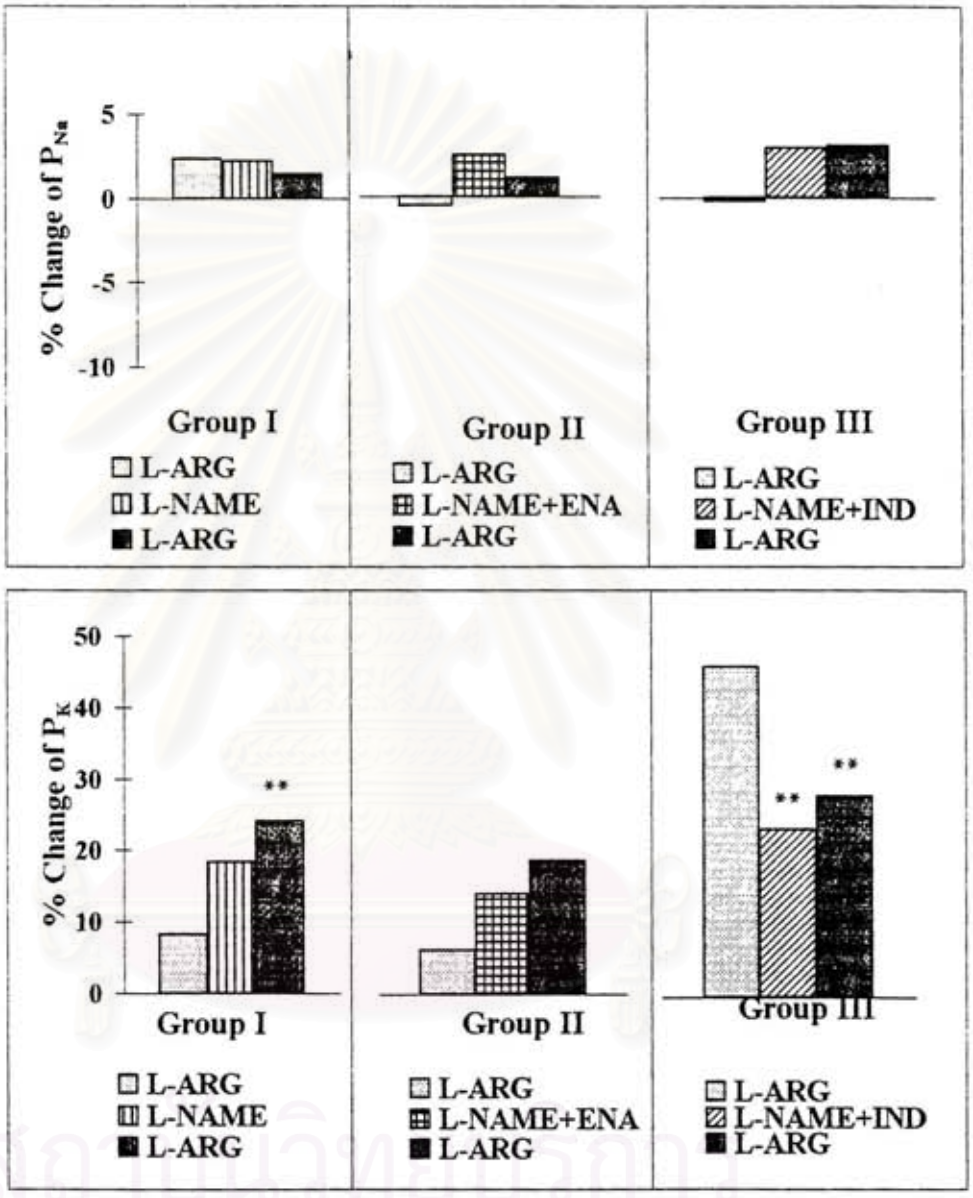


Figure 3 Percentage changes in plasma concentration of sodium (P_{Na}) and plasma concentration of potassium (P_K) in animals given L-arginine loading during administration of L-NAME alone (Group I) ; administration of combination of L-NAME and enalapril maleate (ENA) (Group II) and administration of combination of L-NAME and indomethacin (IND) (Group III).
The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

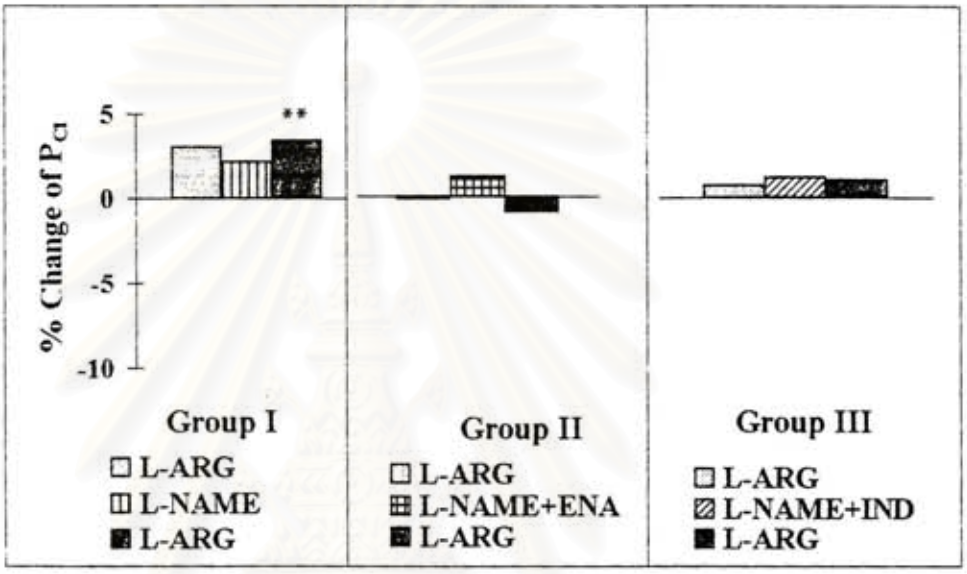


Figure 4 Percentage changes in plasma concentration of chloride (P_{Cl}) in animals given L-arginine loading during administration of L-NAME alone (Group I); administration of combination of L-NAME and enalapril maleate(ENA) (Group II) and administration of combination of L-NAME and indomethacin (Group III).
The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

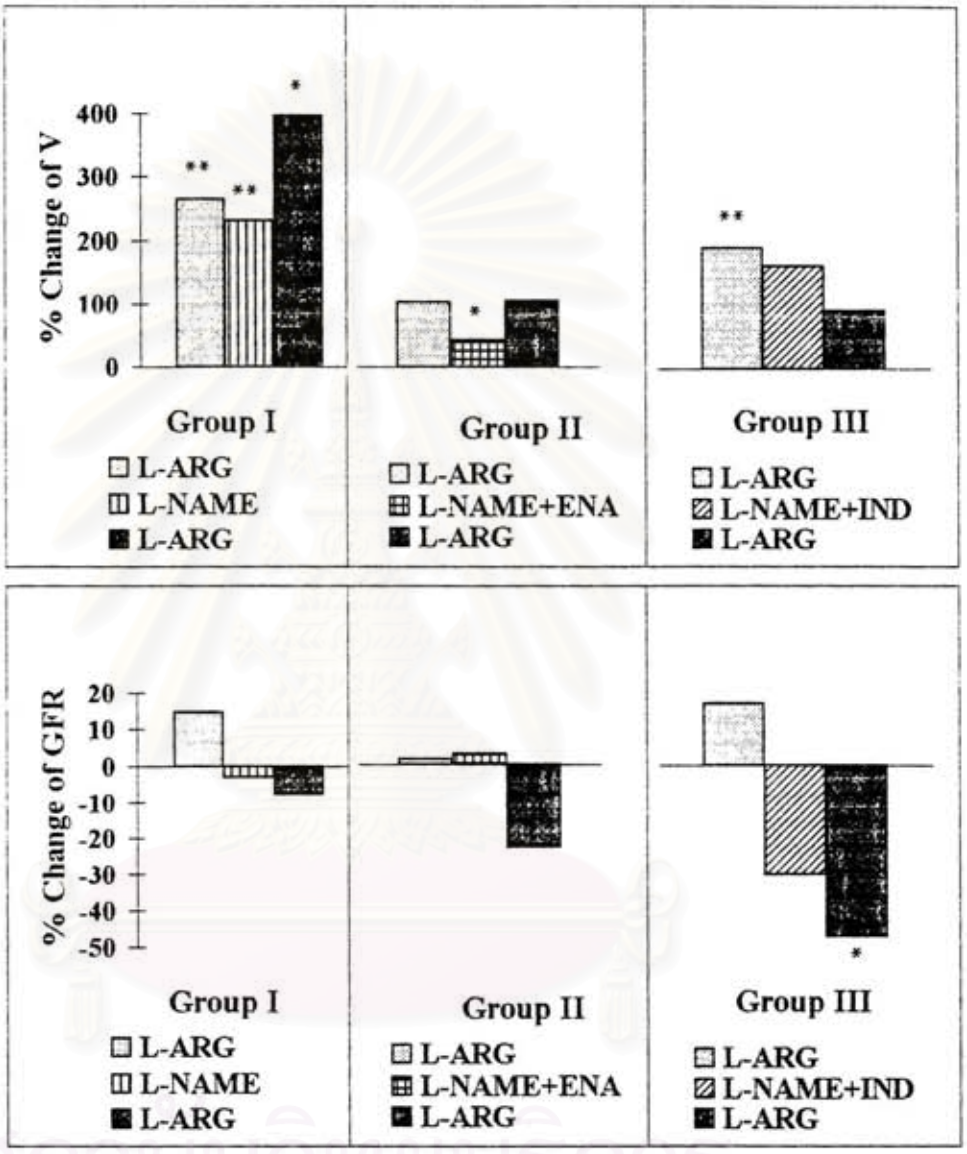


Figure 5 Percentage changes in urine flow rate (V) and glomerular filtration rate (GFR) in animals given L-arginine loading during administration of L-NAME alone (Group I); administration of combination of L-NAME and enalapril maleate (ENA)(Group II) and administration of combination of L-NAME and indomethacin (IND)(Group III). The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

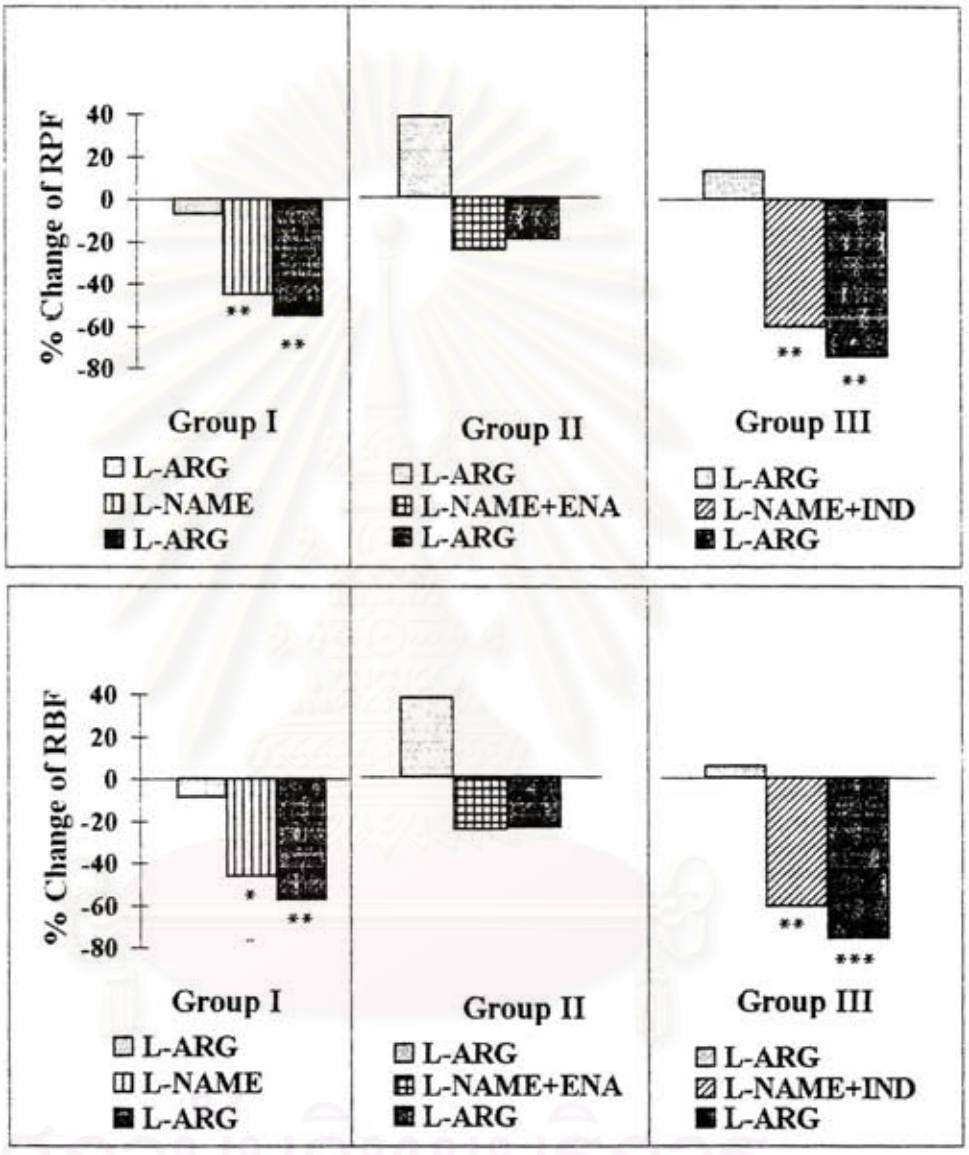


Figure 6 Percentage changes in renal plasma flow (RPF) and renal blood flow (RVR) in animals given L-arginine loading during administration of L-NAME alone(Group I); administration of combination of L-NAME and enalapril maleate (ENA) (Group II) and administration of combination of L-NAME and indomethacin (IND) (Group III). The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

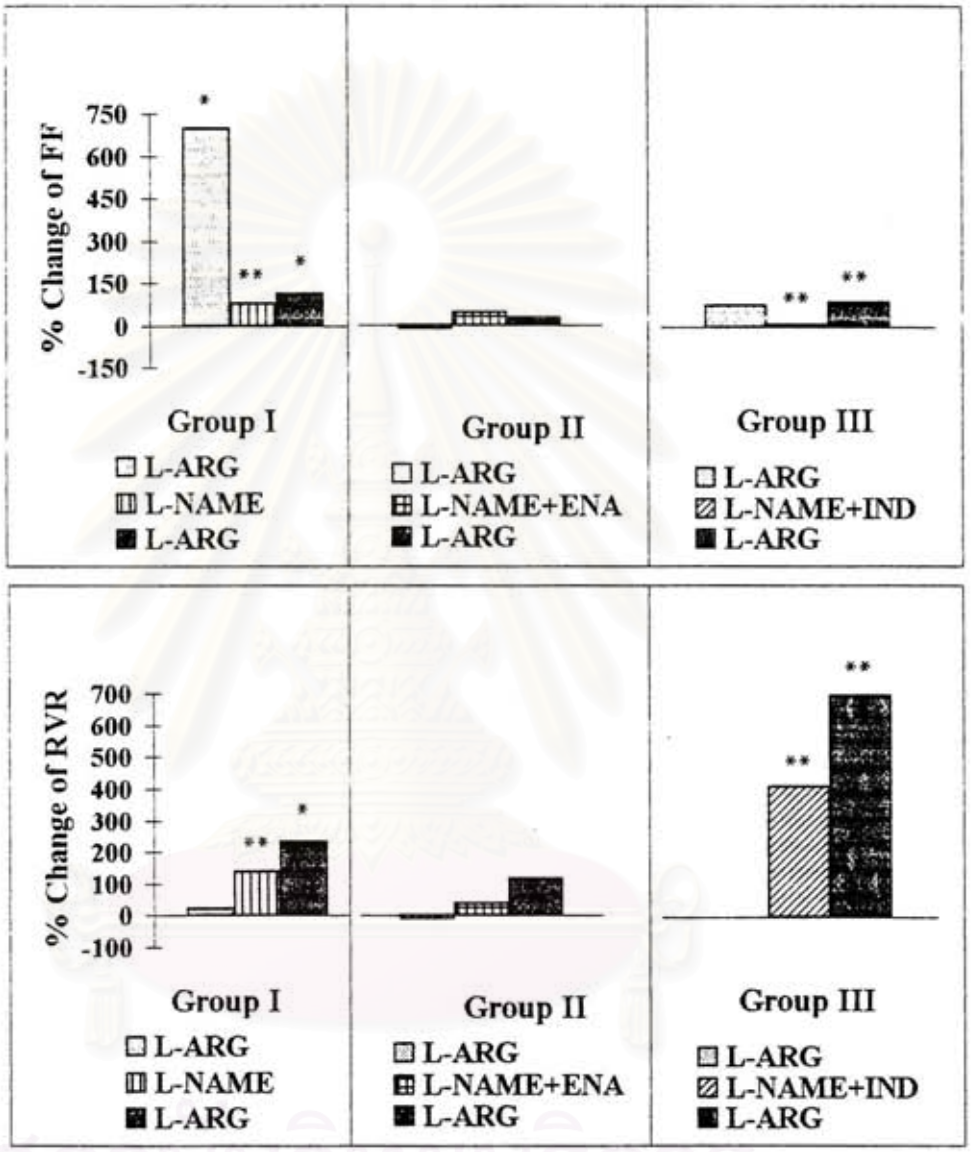


Figure 7 Percentage changes in filtration fraction (FF) and renal vascular resistance (RVR) in animals given L-arginine loading during administration of L-NAME alone (Group I); administration of combination of L-NAME and enalapril maleate (ENA)(Group II) and administration of combination of L-NAME and indomethacin (IND) (Group III).
The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

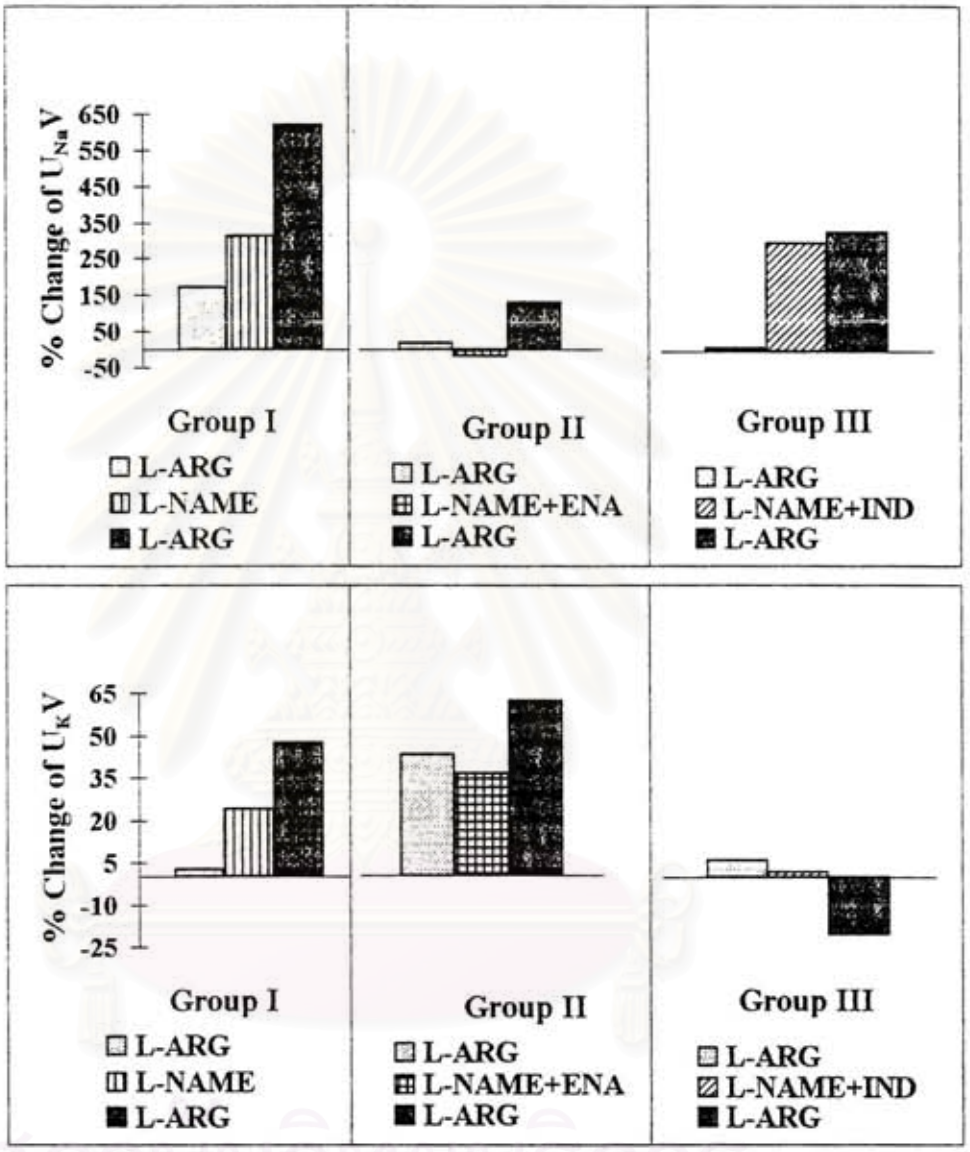


Figure 8 Percentage changes in urinary excretion of sodium ($U_{Na}V$) and urinary excretion of potassium (U_KV) in animals given L-arginine loading during administration of L-NAME alone (Group I) ; administration of combination of L-NAME and enalapril maleate (ENA) (Group II) and administration of combination of L-NAME and indomethacin (IND) (Group III).
The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

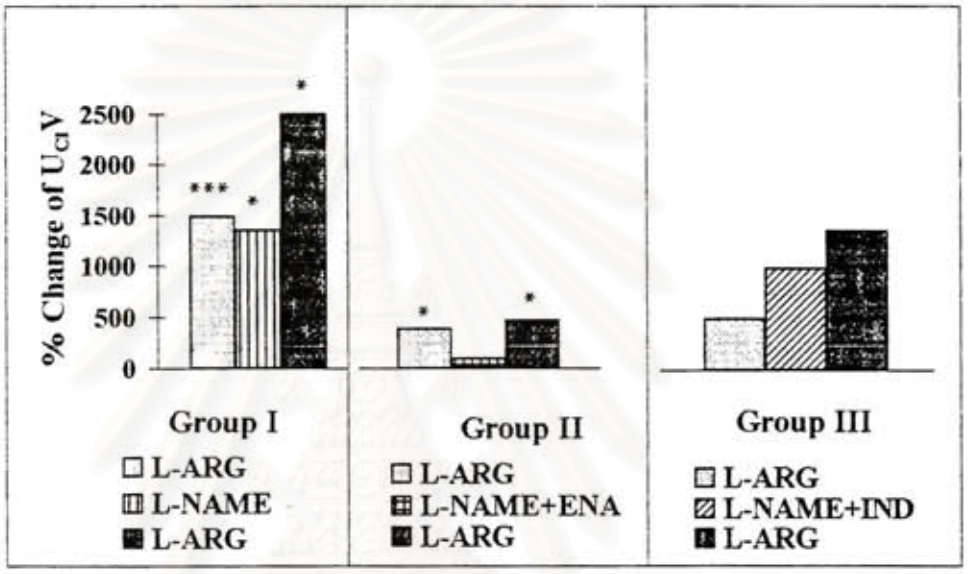


Figure 9 Percentage changes in urinary excretion of chloride ($U_{Cl}V$) in animals given L-arginine loading during administration of L-NAME alone (Group I); administration of combination of L-NAME and enalapril maleate (ENA)(Group II) and administration of combination of L-NAME and indomethacin (IND) (Group III). The value are mean, *P<0.05, **P<0.01, ***P<0.001 compared to control value of each group.

CHAPTER V

DISCUSSION

The purpose of the present study was to investigate the role of L-arginine relating nitric oxide (NO) production on general circulation and renal function in vivo. Whether its physiological roles are mediated via renin-angiotensin or prostaglandin systems using concomitant acute blockade of L-NAME, an inhibitor of the nitric oxide systhesis.

It has been found that HR and MAP were not affected by L-arginine loading alone. However, MAP increased significantly when the anima l was given either L-NAME (NO blockade) alone or L-NAME combination with indomethacin after L-arginine loading. It is known that NO is synthesized from the amino acid, L-arginine by an enzyme, the NO synthase which is produced from the guanidine group of the amino acid L-arginine. NO synthesis can be inhibited by L-arginine competitive analogues, e.g., L-NAME (Rees et al., 1989; Rees et al., 1990 and Bellan et al.,1993). Owing to the physiological importance of the endothetial nitric oxide pathway for relaxation of vascular smooth muscle (Ignarro, L.J., 1990; Palmer et al., 1988). The experiments discussed below focus on the uptake and metabolism of L-arginine, the substrate for the endothelial nitric oxide synthase, and on the tissue content of guanosine 3′5′,-cyclic monophosphate (cGMP), which nitric oxide elevates in smooth muscle cells to mediate relaxation (Ignarro, L.J., 1990; Rapoport et al, 1985a; Rapoport et al, 1985b). The present results suggest that in the anesthetized dogs the general circulation response to NO blockade was not only mediated by prostaglandin system since L-NAME combination with indomethacin produced a greater increase in

MAP. The vasoconstrictor effect of L-NAME can be potentiated by indomethacin. Indomethacin administration has been shown to increase total peripheral resistance (TPR) by reduction vasodilators effect of prostaglandins. An increase in MAP would be due to an increase in vascular resistance following reduce the venous return to the heart, resulting in a reduction of cardiac output (CO) (Braunwald et al., 1979). The potential importance of EDRF (NO) in the maintenance of systemic vascular tone. Endothelium-dependent relaxations occur in resistance arteries where peripheral vascular resistance is regulated (Dohi and Luscher, 1990; Tesfamarian and Halpern, 1988; Dohi et al., 1990; Diederich et al., 1990). However, the change in CO may not be concluded since L-arginine infusion at a higher dose would decrease in TPR and increase in CO which it did not occur in a lower dose (Napathorn et al., 1992). These findings have been suggested that L-arginine infusion induces systemic vasodilatation and increased CO in a dose dependent manner (Napathorn et al., 1992). When the combination of L-NAME and enalapril maleate (ANG II blocker) was administed to the animal, HR and MAP were decreased. Enalapril maleate reduced the action of L-NAME on the general circulation. It indicates from the present results that the general circulation response to NO blockade would be mediated by renin-angiotensin system. This suggests that EDRF (NO) released from endothelial cells inhibits renal renin production. Since endothelial cells response to shear forces exerted by the circulating blood, this response may mediate the renal renin-baroreceptor mechanism (Miller, Redfield and Burnett, 1989; Rakugi et al., 1988). However, the present result was not similar to the experiment in the normal conscious rat by Baylis et al.(1993); which has suggested that acute endothelial derived relaxing factor (EDRF) blockade was not related to endogenous renin-angiotensin system. It is possible that exogenous L-arginine loading before the administration of either combination of L-NAME and enalapril maleate or enalapril maleate alone would increase exogenous precusor of nitric

oxide synthesis (Harald et al., 1988 and Amezcua et al., 1989). Endogenous L-arginine has been reported usually to be a physiological precusor of endothelium-derived nitric oxide (Palmer et al., 1988). Exogenous L-arginine is possible causing vasodilatation. However, many possibility have been reported that the effects on renal hemodynamics after L-arginine loading in the present studies did not show any alterations of GFR, RPF and RVR, but urine flow rate markedly increased. L-arginine is an amino acid can stimulate prostaglandins synthesis on glomerular afferent arteriols (Castellino et al., 1988). A possibility is that renal vasodilatation through a tubuloglomerular feedback (TGF) mechanism, possibly by stimulating proximal tubular sodium chloride reabsorption and that changing the signal at the macula densa (Woods et al., 1986 and Appaini et al., 1988). L-arginine is a large amino acid which can create osmotic gradient similar to change in intrarenal blood flow distribution and the resultant effects on the medullary osmotic gradient and deep nephron function may be involved in pressure natriuresis (Kunau et al., 1976; Selkurt et al, 1965). In case of an increase in the rate of urine flow following L-arginine loading, it is possible that osmotic action of large concentration of the L-arginine by an increase in filtered load led to a fall in fractional reabsorption of sodium. When filtered L-arginine enter Bowman's capsule and flow along the proximal portion of tubules, some solute entering the tubules will be retained within the tubular lumen and create osmotic gradient while interfere with reabsorption of normal fraction of osmotic sodium. Effects of either L-NAME alone or administration of L-NAME combined with indomethacin following L-arginine loading caused decreased in RPF and RBF. RVR and TPR significantly increased while GFR remained constant. It indicates that the inhibitor of nitric oxide administration has been shown to increase both afferent and efferent arterioles resistance (vasoconstriction). The present results have been shown that the inhibitor nitric oxide could affect the renal hemodynamics but not related to the prostaglandins

system. In the kidney, endothelium-derived substances interact with vascular smooth muscle, mesangial, juxtaglomerular and possibly also tubular cells and hence profoundly affect renal hemodynamics and function. It has been known that an increase in NO production related to release of cGMP in the vascular smooth muscle cell (Lahera et al., 1993). In the rat, intravenous infusion of acetylcholine caused hypotension, renal vasodilatation and increase in urinary cGMP excretion, while glomerular filtration rate was maintained (Tolins et al., 1990). On the other hand, L-N^G-monomethyl arginine (L-NMMA) increase renal vascular resistance, reduces urinary cGMP excretion and decreases renal blood flow, while glomerular filtration rate remains constant (Tolins et al., 1990; King et al., 1990). The inhibitor of nitric oxide formation also prevents the renal hemodynamics effects of acetylcholine. Interestingly, L-NMMA also attenuates the vasodilator response to amino acid infusions (King et al., 1990). Thus, endothelium-derived nitric oxide can profoundly affect mesangial cells, juxtaglomerular cells, glomerular function and renal hemodynamics and may act as a local regulatory system of kidney function under physiological conditions. It has been known that many hormones such as prostaglandins (Ruilope et al., 1987 and Hirschberg et al., 1988) would be possible mediators causing renal vasodilatation during amino acid loading (Castellino et al., 1988). The renal hemodynamics changes would be attributed to actions of insulin, growth hormone and glucagon. Tubuloglomerular feedback is another mechanism which has been suggested by many investigators (Woods et al., 1986 and Appaini et al., 1988). During amino acids loading, it could increase proximal sodium reabsorption so the tubuloglomerular feedback was inhibited by reduced distal sodium delivery (Bank et al., 1970). In the present experiment; no significant changes in plasma sodium, potassium and chloride concentration were obtained by any treatments of L-arginine loading alone, L-NAME administration alone, combination of L-NAME and enalapril maleate or combination of L-NAME and indomethacin

administration. In the present studies animals were treated with exception for L-arginine loading alone causing renal vasoconstriction by a marked increased in RVR. These effects could not be reversed when L-arginine given after L-NAME administration or after administration of the combination of L-NAME and indomethacin. These results clearly indicate that L-NAME can be block NO synthesized from L-arginine by inhibited NO synthase. Animals given L-NAME combination with enalapril maleate would decrease in excretions of sodium and chloride. These observations are consistent with the suggestion that action of renin-angiotensin system since action of renin-angiotensin can induce proximal sodium reabsorption (Ohishi et al., 1992). The role of NO relating action renin-angiotensin (ANG II) in the natriuretic/diuretic response to acute EDRF blockade has also been proposed in the conscious rat (De Nicola et al.,1992).

In conclusion of the present studies suggest that a major regulatory role for endogenous EDRF can control of renal hemodynamics in relating nitric oxide production after L-arginine loading. Its physiological roles are partially mediated via renin-angiotensin, but not for prostaglandins.

REFERENCES

Amezcua, L., Plamer, R.M.J., Souza, B.M. de and Moncada, S. (1989) Nitric oxide synthesized from L-arginine regulates vascular tone in the coronary circulation of the rabbit. Br.J.Pharmacol. 97, 1119-1124.

Appiani, A.C., Assael, B.M., Tirelli, A.S., Cavanna, G., Corbetta, C. and Marra, G. (1988) Proximal tubular function and hyperfiltration during amino acid infusion in man. Am.J.Nephrol. 8, 96-101.

Bank. N., Aynedjian, H.S., Bansal, V.K. and Goldman, D.M. (1970) Effect of acute hypertension on sodium transport by the distal nephron. Am.J.Physiol. 219, 275-280.

Baylis. C.K., Kennie, S. and Paul, H. (1993) Renal effects of acute endothelial-derived relaxing factor blockade are not mediated by angiotensin II. Am.J. Physiol. 264 (Heart Circ. Physiol. 33), F74-F78.

Bellan, J.A., Dennis, B.McN and Philip, J.K. (1993) Differential effects of nitric oxide synthesis inhibitors on vascular resistance and response to acetylcholine in cats. Am. J. Physiol. 264 (Heart Circ. Physiol. 33), H45-H52.

Braunwald, E. and Ross, J.Jr. (1979) Control of cardiac performance ; in Berne R.M. (ed.) : Handbook of Physiology. Section 2 : The cardiovascular system. Baltimore, Waverly Press, Inc., 1979, vol.1, pp.539-541.

Burton, G.A.,Macheil, S.,De Jonge, A. and Haylor, J. (1990) Cyclic GMP release and vasodilatation induced by EDRF and atrial natriuretic factor in the isolated perfused kidney of the rat. Br.J.Pharmacol. 99, 364-368.

Castellino, P., Giordona, C., Perna, A. and DeFronzo, R.A. (1988) Effects of plasma amino acid and hormone levels on renal hemodynamics in humans. Am.J. Physiol. 255, F444-F449.

_____..Hunt, W. and De Fronzo, R.A. (1987) Regulation of renal hemodynamics by plasma amino acid and hormone concentrations. Kidney Int. 32 (Suppl. 22), S15-S20.

Chaiyabutr, N., Faulkner, A. and Peaker, M. (1980) Effects of starvation on the cardiovascular system, water balance and milk secretion in lactating goat. Res. Vet. Sci. 28, 291-295.

De Nicola. L., Blantz, R.C. and Gabbai, F.B. (1992) Nitric oxide and angiotensin II glomerular and inbular interaction in the rat. J. Clin. Invest. 89, 1248-1256.

Deng, A. and Baylis, C. (1993) Locally produced EDRF controls pre-glomerular resistance and ultrafiltration coefficient. Am.J.Physiol. 264 (Renal Fluid Electrolyte Physiol.33), F212-F215.

Diederich, D., Yang, Z., Bühler, F.R. and Lüscher, T.F. (1990) Impaired endothelium-dependent relaxations in hypertensive mesenteric resistance arteries involve cyclooxygenase pathway. Am. J. Physiol. 258, H445-H451.

Dohi, Y. and Lüscher, T.F. (1990) Hypertension differentially affects intraluminal and extraluminal activation of the endothelium. (abstract) Hypertension 16, 315.

_____., Thiel, M., Bühler, F.R. and Lüscher, T.F. (1990) Activation of endothelial L-arginine pathway in resistance arteries : Effect of age and hypertension. Hypertension 15, 170-175.

Featherston, W.R., Rogers, Q.R. and Freedland, R.A. (1973) Relative importance of kidney and liver in synthesis of arginine by the rat. Am.J.Physiol. 224, 127-129.

Furchgott, R.F. (1983) Role of the endothelium in responses of vascular smooth muscle. Cirs.Res. 53, 557-573.

_____., Vanhoutte, P.M. (1989) Endothelium-derived and contracting factors. FASEB J., 2007-2018.

Guler, H.P., Eckardt, K.U., Zapf, J., Bauer, C. and Froesch, R. (1989) Insulin-like growth factor I increases glomerular filtration rate and renal plasma flow in man. Acta Endocrinol. 121, 101-106.

Harald, H.H.W.. Schmidt, H.N., Werner, W., Jorg, G., Karl-Ernst, P., Marcus, M.K., Feraydoon, N.and Eycke, B. (1988) Arginine is a physiological precusor of endothelium-derived nitric oxide. Eur. J. Pharmacol. 154, 213-216.

Hecker. M., Sessa, W.C., Harris, H.J., Annggard, E.E.and Vane, J.R. (1990) The metabolism of L-arginine annd its significance for the biosynthesis of endothelium-derived relaxing factor : cultured endothelial cells recycle L-citrulline to L-arginine. Proc.Natl.Acad.Sci. USA. 87, 8612-8616.

Hirschberg, R.R., Zipser, R.D., Siomowitz, L.A. and Kopple, J.D. (1988) Glucagon and prostaglandins are mediators of amino acid-induces rise in renal hemodynamics. Kidney Int. 33, 1147-1155.

Hoffend, J.,Cavarape, A.,Endlich, K. and Steinhansen, M. (1993) Influence of endothelium derived relaxing factor on renal microvessel and pressure-dependent vasodilation. Am.J.Physiol. 265 (Renal Fluid Electrolyte Physiol. 34), F285-F292.

Ignarro, L.J. (1989) Biological actions and properties of endothelium-derived nitric oxide formed and released from artery and vein. Circ.Res. 65, 1-21.

_____. (1990) Nitric oxide : a novel signal transduction mechanism for transcellular communication. Hypertension 16, 477-483.

_____.,Kadowitz, P.J. (1985) The pharmacological and physiological role of cyclic GMP in vascular smooth muscle relaxation. Annu. Rev. Pharmacol.Toxicol 25, 171-191.

Johannesen, J., Lie, M. and Kiil, F. (1977) Effect of glycine and glucagon on glomerular filtration and renal metabolic rates. Am.J.Physiol. 233, F61-F66.

King, A.J., Troy, J.L., Downes, S.J., Anderson, S. and Brenner, B. (1990) Effects of N^G-monomethyl-L-arginine (L-NMMA) on basal renal hemodynamics and the response to amino acid (AA) infusion. (abstract) Kidney Int. 37, 371.

Kunau, R.T. and Lameire, N.H. (1976) The effect of an acute increase in renal perfusion pressure on sodium transport in the rat kidney. Circ. Res. 39, 689-695.

Lahera, V., Salom, M.G., Miranda-Guardiola, F., Moncada, S., and Romero, J.C. (1991) Effects of N^G-nitro-L-arginine methyl ester on renal function and blood pressure. Am.J. Physiol. 261. (Renal Fluid Electrolyte Physiol. 28), F1033-F1037.

_____.,Navarro, J.,Biondi, M.L.,Ruilope, L.M. and Romero, J.C. (1993) Exogenous cGMP prevents decrease in diuresis and natriuresis induced by inhibition of NO synthesis. Am.J.Physiol. 264 (Renal Fluid Electrolyte Physiol.33), F344-F347.

Levillain, O., Hus-Citharel, A., Morel, F. and Bankir, L. (1990) Localization of arginine synthesis along rat nephron. Am.J.Physiol. 259, F916-F923.

Miller, W.L., Redfield, M.M. and Burnett, J.C. (1989) Integrated cardiac, renal, and endocrine actions of endothelin. J. Clin. Invest. 83, 317-320.

Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1989) Biosynthesis of nitric oxide from L-arginine : a pathway for the regulation of cell function and communication. Biochem. Pharmacol. 38, 1709-1715.

Napathorn, S., Chaiyabutr, N., Buranakarl, C., Pansin, P., Pochanugool, C., Sridama, V. and Sitprija, V. (1992) Effects of acute arginine loading on renal and systemic hemodynamics in dogs. Nephron. 60, 220-225.

Ohishi, Kazuhisa, Pamela K. Carmines, Edward W. Inscho and L. Gabriel Navar (1992) EDRF-angiotensin II interactions in rat juxtamedullary afferent and efferent arterioles. Am.J. Physiol. 263 (Renal Fluid Electrolyte Physiol.32), F900-F906.

Palmer, R.M.J., Rees, D.D., Ashton, D.S. and Moncada, S. (1988) L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biophys. Res. Commun. 153, 1251-1256.

Radermacher, J., Bernd, K., Sabine, K., Gerold, H., Hams, J.S., Hilmer, F.S. and Jurgen, C.F. (1991) Effect of arginine depletion on glomerular and tubular kidney function : studies in isolated perfused rat kidneys. Am.J.Physiol. 261 (Renal Fluid Electrolyte Physiol. 30), F779-F786.

_____..Forstermann, U. and Frolich, J.C. (1990) Endothelium-derived relaxing factor influences renal vascular resistance. Am.J.Physiol. 259 (Renal Fluid Electrolyte Physiol. 28), F9-F17.

_____..Klanke, B. ,Kastner, S. ,Haake, G. ,Schurek, H.J., Ruilope, L.M., Rodicio, J., Robles, R.G.,Sancho, J., Miranda, B.,Granger, J.P., Romero, J.C. (1987) Influence of a low sodium diet on the renal response to amino acid infusions in humans. Kidney Int. 31, 992-999.

Rakugi, H., Nakamaru, M., Saito, H.,Higaki, J., Ogihara, T. (1988) Endothelin inhibits renin release from isolated rat glomeruli. Biochem. Biophys. Res. Commun. 155, 1244-1247.

Rapoport, R.M., Schwartz, K., and Murad, F. (1985) Effects of Na^+, K^+-pump inhibitors and membrane depolarizing agents on acetylcholine induced endothelium-dependent relaxation and cyclic GMP accumulation in rat aorta. Eur. J. Pharmacol. 110, 203-209.

_____.,Schwartz, K., and Murad, F. (1985) Effects of sodium-potassium pump inhibitors and membrane depolarizing agents on sodium nitroprusside induced relaxation and cyclic guanosine monophosphate accumulation in rat aorta. Circ. Res. 57. 164-170.

Rees, D.D., Palmer, R.M.J., Hodson, H.F. and Moncada, S. (1989) A specific inhibitor of nitric oxide formation from L-arginine alternates endothelium-dependent relaxation. Br.J. Pharmacol. 96, 418-424.

_____..Palmer, R.M.J., Schultz, R. and Hodson, H.F. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro, and in vivo. Br.J. Pharmacol. 101. 746-752.

Ruilope, L.M., Rodicio, J., Robles, R.G., Sancho, J., Miranda, B., Granger, J.P. and Romero, J.C. (1987) Influence of a low sodium diet on the renal response to amino acid infusion in humans. Kidney Int. 37, 992-999.

Selkurt, E.E., Womack, I. and Dialey, W.N. (1965) Mechanism of natriuresis and diuresis during elevated renal arterial pressure. Am. J. Physiol. 209, 95-99.

Sessa, W.C., Hecker, M., Mitchell, J.A. and Vane, J.R. (1990) The metabolism of L-arginine and its significance for the biosynthesis of endothelium-derived relaxing factor : L-glutamine inhibits the generation of L-arginine by cultured endothelial cells. Proc. Natl. Acad. Sci. USA 87, 8607-8611.

Smith, H.W. Principle of renal physiology. London : Oxford University Press, 1962.

Snegoski, M.C., and Freier, E.F. An automated o-toluidine glucose procedure without acetic acid. Am.J.Med.Technol. 39 (1973) : 140-150.

Tesfamerian, P. and Halpern, W. (1988) Endothelium-dependent and endothelium-independent vasodilatation in resistance arteries from hypertensive rats. Hypertension 11, 440-444.

Tolins, J.P., Palmer, R.M.J., Moncada, S. and Raij, L. (1990) Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. Am. J. Physiol. 258, H655-H662.

Visek, W.J. (1985) Arginine and disease states. J.Nutr. 115, 532-541.

Walder, C.E., Thiemermann. C. and Vane, J.R. (1991) The involvement of endothelium-derived relaxing factor in the regular of renal cortical blood flow in the rat. Br.J.Pharmacol. 102, 967-973.

Windmueller, H.G., and Spaeth, A.E. (1981) Source and fate of circulating citrulline. Am.J.Physiol. 241 (Endocrinol. Metab. 4), E473-E480.

Woods, L.L., Mizelle, H.L. and Hall, J.E. (1987) Role of liver in renal hemodynamic response to amino acid infusion. Am.J.Physiol. 252, F981-F985.

_____.,Mizlle, H.L., Montani, J.P. and Hall, J.E. (1986) Mechanisms controlling renal hemodynamics and electrolyte excretion during amino acids. Am.J.Physiol. 251, F303-F312.

Young, M.K., and Raisz, I.G. An anthrone procedure for determination of inulin in biological fluids. Proc.Soc.Exper.Biol. and Med. 80 (1952) : 771-774.

BIOGRAPHY

Miss Chutima Udom was born on 29th July, 1966 and graduated with the Bachelor of Science in Nursing from Hau Chew College in 1987, now that place changes to call "Hua Chew Chaleamprakeat University". She admitted with the degree of Master of Science, Inter-Department of Physiology, Chulalongkorn University in 1991.