NEUROPEPTIDES IN THE CONTROL OF HYDROMINERAL BALANCE AND BLOOD FLOW

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TO MY FAMILY

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ABBREVIATIONS

ADO	Adenosine
ANG II	Angiotensin II
A_3V	Anterior third ventricle
AVP	Arginine vasopressin
ANP	Atrial natriuretic peptide
CGRP	Calcitonin-gene-related peptide
CVO(s)	Circum-ventricular organ(s)
CSF	Cerebrospinal fluid
EDRF	Endothelium-derived relaxing factor
IA	Intra-arterial
ICV	Intracerebroventricular
-IR	Immunoreactive
IV	Intravenous
NO	Nitric oxide
L-NAME	L-nitro arginine methyl ester
L-NMMA	L-nitro mono methyl arginine
NP	Neuropeptide
NPY	Neuropeptide Y
NE	Norepinephrine
ORS(T)	Oral rehydration salt (therapy)
OVLT	*Organum Vasculosum of the Lamina Terminalis*
SFO	Subfornical Organ
SP	Substance P

ABSTRACT

Electrical stimulation of the sciatic nerve (2 Hz, 1 ms, 3 v, 40 pulses at 5 min intervals) increased hind limb vascular resistance in rabbits. However, when guanethidine was applied the nerve stimulation induced a decrease in hind limb resistance and an increase in hind limb blood flow which was pulse duration-, frequency- and voltage dependent. The nerve induced responses were not affected by cholinergic or adrenergic blockade.

The nerve induced vasodilatory responses were mimicked by local intra-arterial infusions of substance P, both of which could be diminished by L-NAME (a nitric oxide synthesis blocker). In another set of experiments, CP-96,345, a highly selective antagonist of neurokinin-1 receptor, diminished both the nerve-induced as well as the substance P-induced decreases in hind limb vascular resistance. Neither L-NAME nor CP-96,345 affected the vasodilatory responses elicited by adenosine.

In another set of experiments in sheep, L-NMMA (another nitric oxide synthesis blocker) was infused into the lateral ventricles. NO blocking centrally was associated with an increase in free water clearance and a decrease in plasma AVP levels during the infusion. Blood pressure decreased and heart rate increased during the infusion. D-NMMA had little effect.

In a similarly prepared sheep, as the previous experiment, water deprivation for 96 hours resulted in a significant increase in plasma AVP and ANG-II levels as well as a significant increase in the levels of cerebrospinal fluid ANG-II, NPY and CGRP levels all of which paralleled to an increase in plasma osmolality. The levels of the neuropeptides decreased when water was allowed and animals hydrated.

The last study was conducted in thirteen children with moderate to severe degree of dehydration secondary to diarrhoea who were admitted to the ORT centre at Ethio-Swedish Paediatric hospital for rehydration with ORS. Plasma samples taken before the start of ORS therapy and another sample collected after full hydration were compared for various variables. Plasma osmolality, sodium and potassium were lower than controls before ORS therapy all of which increased after full hydration was achieved. Total plasma protein, measured to assess the state of hydration, decreased after the ORS therapy.

The results suggest the importance of the above mentioned neuropeptides in hydromineral balance and blood flow control in mammals. The involvement of the L-arginine/ NO pathway in blood flow is also demonstrated and the possible central involvement of this pathway in hydromineral balance and release of some neuropeptides is suggested.

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

A. Nitric Oxide in Modulation of Blood Flow and Hydromineral Balance.

Endothelium-derived relaxing factor (EDRF) has been shown to be nitric oxide (NO) derived from L-arginine in many studies by NO-synthase (Palmer *et al*, 1987; Toda and Okamura, 1990; Moncada *et al*, 1991). The exact mechanism by which the terminal guanido-nitrogen atom of L-arginine is liberated and subsequently oxidized to NO is not clearly known and three types of NO-synthase in different tissues have been described (Rees *et al*, 1990; Xie *et al*, 1992). However this reaction needs soluble enzymes, a divalent cation and is NADPH dependent (Ralevic *et al*, 1991).

NO is a gas, soluble in aqueous medium and functions as a molecule in solution. The enzymatic reaction that forms NO from L-arginine is quite specific. L-NMMA, L-NAME or other analogues of L- arginine inhibit NO synthesis in a dose dependent manner, while their D-counterparts are without effect (Rees *et al*, 1990, Vila *et al*, 1990). NO is not very reactive at neutral pH but its paramagnetic property gives it a high binding affinity for haem, reacting with haemo-proteins to generate their nitrosyl haem adducts, which are more stable than free NO (Moncada *et al*, 1991). The other bi-product is L-citrulline (Lowenstein and Snyder, 1992).

The exact source of L-arginine is not clearly identified but there is a suggestion that it may be obtained either from the diet or synthesized from L-glutamate in the urea cycle (Moncada *et al*, 1991).

Nitric oxide once formed, diffuses out of its cells of origin to nearby target cells and binds to the haem part of cytosolic guanylate cyclase causing its activation (Moncada *et al*, 1989; Lowenstein and Snyder, 1992). Because of its molecular size and lipophilic nature NO becomes a very good candidate for trans-cellular communication, but because of its short half-life which is less than five seconds, the effect is limited to localized responses and makes it unlikely to serve as a long acting messenger (Moncada *et al*, 1989; Ignarro, 1990). At the molecular level NO stimulates soluble guanylate cyclase and its function is associated with a rise in cellular cGMP levels (Ingrid *et al*, 1991).

The L-arginine/NO pathway is involved in a wide variety of tissue functions. In vascular system NO causes vasodilation and inhibits local platelet aggregation (Toda and Okamura, 1991). This pathway is also involved in other cells such as macrophages (Palmer *et al*, 1987, Xie *et al*, 1992) and neutrophils (Moncada *et al*, 1991) aiding in the cytotoxic function of these cells. NO is also suggested to have an important role for cell to cell communication in the central nervous system (Ignarro, 1990).

One of the most well studied and established functions of NO is in the vascular systems where there is a continuous utilization of L-arginine for the generation of nitric oxide. NO plays an important role in the maintenance of blood flow (Ignarro, 1990; Bult *et al*, 1990; Antonia and Burnstock, 1991). Reduction in NO production has been implicated in some cases of essential hypertension (Ignarro, 1990) and by modulating mesangial cell contractility, NO is thought to modify renin release there by affecting blood pressure (Ignarro, 1990). NO has also been shown to act as a local blood flow regulator preventing thrombosis and

maintaining blood flow to vital organs by keeping the patency of these vessels (Moncada *et al*, 1991; Toda and Okamura, 1991). Since NO could not explain the vasodilator responses caused by EDRF in some vascular tissues, the existence of other labile nitroso-compounds has been suggested (Toda and Okamura, 1991; Moncada *et al*, 1991).

The actions of vasodilator and vasoconstrictor peptides have been studied in relation to NO in many mammalian species (Vila *et al*, 1991; Ralevic *et al*, 1991). Toda and collaborators (1990) studied the effect of transmural vasodilator nerve stimulation in isolated cerebral arteries of dog and monkey. The vasodilator response was abolished by tetrodotoxin and hexamethonium and suppressed by L-NMMA, an inhibitor of NO synthesis. The inhibitory effect of L-NMMA was reversed and prevented by L-arginine but not by D-arginine. The effect of nicotine was also assessed in this study and it was found that chemical or electrical stimulation of vasodilator nerves relaxed cerebral arteries of dog and monkey by releasing NO.

In another investigation blood vessels taken from human omentum during operation were studied (Ralevic *et al*, 1991). Arterial rings which were preconstricted by NE were dilated by acetylcholine (Ach) and sodium nitroprusside. Application of L-NMMA or L-NAME or removal of the endothelium inhibited the responses to Ach, but did not affect those responses to sodium nitroprusside, which had a direct vasodilator effect. Studies in vascular rings from human internal mammary artery and vein have also shown that the endothelium-dependent relaxation in these vessels is due to NO. Relaxation by Ach in the previous study using omental vessels was not totally inhibited by the two L-arginine analogues

suggesting the existence of other endothelium-dependent vasodilators other than NO. Prostacyclin is suggested in this respect (Ralevic *et al*, 1991). Unlike the case in rabbits (Gustafsson *et al*, 1990) application of L-NMMA in mesenteric vessels taken from human omentum had no direct vasoconstrictor effect (Vila *et al*, 1991; Ralevic *et al*, 1991). This could be due to species or anatomical differences in the basal rate of NO production, thus a low basal production of NO in mesenteric vessels could have made them less sensitive to L-arginine analogues.

In isolated and perfused rat mesenteric and rabbit hepatic arteries application of sodium nitropruside and Ach caused vasodilation in both types of vessels (Ralevic *et al*, 1991). L-NAME attenuated the response of Ach but enhanced the relaxant effect of sodium nitropruside. In other studies as well (Villa *et al*, 1991) NO-synthase inhibitors enhance the vasodilator response of femoral, coronary and basilar arteries to nitro-vasodilators. This was shown to be due to inhibition of endogenous NO production, which would otherwise consume more of the soluble guanylate cyclase used by nitro-vasodilators. An upward-regulation of the receptors was also found in these conditions (Lowenstein and Snyder, 1991).

The effect of NO in relation to non-adrenergic and non-cholinergic (NANC) neurotransmission in smooth muscles was studied by Bult and collaborators (1990). In this study transmural nerve stimulation in the ileum and pulmonary artery caused ileal contraction which was enhanced by L-NMMA. In other related studies L-NMMA partially inhibited relaxation of guinea pig smooth muscle (Gustafsson, *et al*, 1991; Demman *et al*, 1991). Endogenous NO-like activity was previously documented in the guinea pig. In all of the studies mentioned above, all analogues of L-arginine used inhibited the nerve-induced relaxation in a concentration-dependent manner.

The effects of the analogues were reversed by L-arginine but not by D-arginine. The relaxation induced by nerve stimulation which was inhibited by L-NMMA, was also inhibited by spantide, a substance P antagonist. Relaxations caused by ATP, nitroprusside or bradykinin were not affected by L-NMMA (Gustafsson *et al*, 1990). The responses that occur during stimulation of the NANC nerves in the canine lower oesophageal sphincter were shown to be mediated by NO or an NO releasing substance (Demman *et al*, 1991) which was also demonstrated in guinea pigs (Gustafsson *et al*, 1990).

Besides the various functions discussed above the L-arginine/NO pathway is also involved in other cellular functions. Administration of L-arginine causes release of growth hormone, prolactin, insulin, glucagon, pancreatic somatostatin, pancreatic polypeptide, adrenal catecholamines and vasopressin, in different species, both *in vitro* and *in vivo* (Moncada *et al*, 1991). NO has also been implicated in smooth muscle cell replication and regulation of enzymes involved in the metabolism of cholesterol (Ignarro,1990).

Vasoactive peptides, vasoconstrictors or vasodilators, act directly on smooth muscles or via the release of certain endothelium derived factors which either stimulate or inhibit vascular tone (Bult *et al*, 1990). Vascular relaxations by SP, neurokinin A or B are inhibited by the removal of endothelium or by the application of L-NMMA in a concentration dependent manner (Vila *et al*, 1990, Ralevic, 1991). In 1985, D'Orleans-Juste and his group reported that several peptides act as vasoconstrictors or vasodilators by stimulating or inhibiting the releases of EDRF, which was latter shown to be nitric oxide. Among the various peptides studied, neurokinins and kinins are the most potent vasodilators that use the L-arginine/NO

pathway (Regoli *et al*, 1990). Antonia and co-workers (1991) studied the endothelium dependent and endothelium independent vasodilatation in the rabbit hepatic artery. It was found that noradrenaline contracted hepatic arterial segments and its action was not affected by the presence or absence of the endothelium. Acetylcholine relaxed pre-constricted arteries by NE and necessitated the presence of the endothelium.

Immuno-histochemical studies showed the presence of nerves containing SP, CGRP and VIP in the hepatic artery. SP caused concentration-dependent relaxation of these arteries entirely dependent on the endothelium, whereas CGRP and VIP caused relaxations which were independent of the endothelium, suggesting that the last two peptides were acting on vascular smooth muscle directly while SP was acting on the endothelium. This and other studies show that, besides the classical sympathetic and para-sympathetic control of vascular tone, both peptidergic and purinergic controls are also important. ATP which is released with NE, acts directly on smooth muscles to cause vasoconstriction, with the exception of the rabbit hepatic artery which has a different receptor.

The effect of various neuropeptides in relation to NO in blood flow changes induced by nerve stimulation is not well established. Whether NO is acting as modulator to these peptides or whether it is a primary neurotransmitter of NANC neurons released from the nerves has yet to be proved.

B. Neuropeptides in Hydromineral Balance and Blood Flow Control.

The concept of the *constancy of the internal environment*, as depicted by the French physiologist Claude Bernard, more than hundred years ago, has ever since had a great impact on the thinking in terms of regulatory processes. Some fifty years later, the American Scientist W.B Cannon coined the expression *homeostasis* for the multiple steady states of body functions. Already at this stage it was quite clear that both neural and hormonal control mechanisms are involved in most regulatory processes of living organisms. Somewhat later Scharrer & Scharrer and Bargmann (Bargmann & Scharrer, 1951) discovered that nerves can secrete a humeral substance into the blood which was called *neurosecretion*. This created a link between the nervous and the endocrine system. It subsequently became apparent that the majority of substances released in this manner are peptides, and during that last couple of decades a vast number of neuropeptides have been identified in neural and other tissues. This group of substances, ranging from only a few up to over a hundred of amino acid residues, appear to be involved in many body functions such as reproduction, growth, learning and behaviour.

Among the various neuropeptides that are involved in fluid and electrolyte balance control and cardiovascular function in mammals, arginine vasopressin (AVP), Angiotensin II (ANG II), atrial natriuretic peptide (ANP) and substance P (SP) are well known and rather extensively studied. However, the recently discovered peptides calcitonin-gene-related peptide (CGRP) and neuropeptide Y (NPY) may also play important roles in this respect. Besides the behavioral effects some of these peptides elicit in experimental animals, they appear to play roles in the maintenance of fluid and electrolyte balance and blood flow control to vital organs during various states of hydration. The experiments reported in this thesis look into some aspects of these possible functions.

1. ARGININE VASOPRESSIN (AVP)

The history of AVP goes back to the end of the last century. In 1895 Oliver and Schaefer first observed that pituitary extracts had pressor activity, and a few years later, Howell (1898) found that the pressor material resides in the posterior lobe. A decade later the water conserving properties of neurohypophyseal extracts were discovered, when Farini in Italy and Vand den Velden in Germany successfully treated patients with central diabetes insipidus by injections of such extracts. Experimental evidence for the antidiuretic effect of AVP was obtained in 1924 by Starling and Verney (Lazlo *et al*, 1991). At this time, the vasoconstrictor effect of AVP was also directly observed by Krogh when applying neurohypophyseal extracts on the web feet of frogs. During the following decades, the principle of neurosecreation was discovered by studying the hypothalamo-neurohypophyseal system (Bargmann & Scharrer, 1951) and several aspects of the regulation of AVP release were elucidated (see below). However, the amino acid sequence of AVP was not determined until the 1950's. Since then, sensitive and specific radio-immunoassay methods for determination of the peptide have been developed, which together with molecular biology methods, have enabled an extended knowledge about the regulation of its release as well as synthesis in physiological and pathophysiological situations.

Arginine vasopressin is an evolutionary well preserved peptide present in most mammals. In some species arginine is substituted for lysine at position eight. Amphibians and birds have a closely related peptide. The classical function of AVP is its involvement as a hormone in water homeostasis. As such, it is released from the neuro-hypophysis where it is 'stored' in the nerve endings of the neurosecretory

cells of the supraoptic (SON) and paraventricular (PVN) nuclei in the hypothalamus. AVP may also have other regulatory functions. There are projections from the PVN to the median eminence, where AVP is released into the portal vessels, and participate in the control of ACTH release. In addition, AVP has been suggested to be involved as a neuromodulator in neuronal circuits controlling learning, memory, and thermoregulation (McKinley *et al*, 1983). Immuno-cytochemical studies have also demonstrated projections from the PVN and SON to the wall of the third ventricle, suggesting possible release directly to the CSF (Simon-Opperman *et al*, 1980; Szcepanska-Sadowska *et al*, 1983).

The basal plasma concentration of AVP in most mammalian species is in the range of 1-4 pmol/l, and the plasma $t_{1/2}$ is only a couple of minutes. The plasma levels are positively correlated to the tonicity of the extracellular fluid, and negatively correlated to the volume of that fluid compartment. The release of AVP could also be affected by drugs, emotional stress, pain, nausea etc. (Bie, 1980; Share *et al*, 1988; Janiak and Brody, 1988).

The vasopressor property of AVP may come into play in cardiovascular control in certain situations. So far two different receptors (V1 and V2) have been identified which mediate the effects of AVP. The receptors on vascular smooth muscle and renal mesangial cells are of the V1, whereas those in renal tubular epithelium mediating antidiuretic effects are of the V2 type (Takeda *et al*, 1988; Walker *et al*, 1988). The V1 receptors are coupled to inositol phosphate signal transduction mechanisms, while V2 receptors activate the adenylate cyclase system (Steiner and Phillips, 1988). Little is known about possible regulation of these

receptors, but it has been reported that the V2 receptors may be down-regulated during dehydration (Steiner & Phillips 1988).

The physiological control of AVP release is mainly governed by influences of both the composition (so called osmotic control) and volume (so called volumetric control) of the extracellular fluid. Regarding the former, the classical studies by Verney (1947) in dogs formed the basis for the 'osmo-receptor' concept, i.e. that sensors, whose activity are determined by their own cell volume. This has been supported in many experimental studies in other species, and the idea has gained widespread acceptance (Robertson, 1985).

More recent investigations have provided some evidence for these sensors to be located in circumventricular organs, i.e. structures that lack an effective blood brain barrier (BBB) (McKinley, 1985). However, there is some evidence from studies in ruminant and other species that 'osmo-sensors' may also be located inside the BBB, and that they appear specifically sensitive to the extracellular Na^+ concentration, rather than tonicity (Andersson, 1978). Nevertheless, in most situations variations in body fluid tonicity will be caused by changes in NaCl concentration. The 'osmotic' influence on the AVP release is the major determinant of day to day influences on the plasma concentration of the hormone. This control is very sensitive and a deviation from normal plasma tonicity by about one per cent is sufficient to alter AVP secretion with subsequent changes in urinary concentration and flow (Robertson, 1985). Strong osmotic stimuli increase the plasma AVP concentration to levels around 10-15 pmol/l. The effects of the extracellular fluid volume on the AVP secretion appear less sensitive. Here, about 5-10% deviation from normal is needed to obtain a significant change in AVP levels.

On the other hand, hypovolemia and/or hypotension may elevate the plasma hormone levels several hundred-fold, and hypotensive haemorrhage is the most potent stimulus known. The influence of the ECF volume seems to involve both humeral and reflex links. Hypovolemia activation of the renin angiotensin system (RAS) stimulates AVP secretion via direct central actions of angiotensin II (ANG II). This effect of ANG II, as well as its dipsogenic, hypertensive and natriuretic effects, is dependent on the prevailing extracellular Na^+ concentration (Andersson, 1978). Regarding the reflex link of the 'volumetric' control, arterial baroreceptors, as well as cardiopulmonary distension receptors, exert a tonic inhibitory influence on the AVP release. Thus, the high plasma levels in response to hypotension, as mentioned above, are caused by a decreased inhibitory input from these cardiovascular senors (Robertson, 1985).

It is also apparent that hypovolemia/hypotension exerts a stronger (stimulatory) influence on the AVP secretion than is seen during hypervolemia/ hypertension (inhibitory). The 'osmotic' and 'volumetric' control of AVP release may interact in certain situations when synergistic or opposing influences may be at hand. Dehydrated (Water deficient) subjects are both hyperosmotic and the plasma AVP concentration may rise to levels above 20 pmol/l depending on the degree of concurrent hypovolemia. During mild to moderate degrees of hypertonic dehydration the plasma osmolality has been found to account for about 75% of the stimulus for AVP release (Woods and Johnstons, 1983).

When dehydrated animals and humans rehydrate themselves by drinking water, the AVP release is reflexly inhibited, long before any change in plasma osmolality (Davison *et al*, 1988). The response appears to be elicited at the

oropharyngeal level, and several characteristics of the ingested fluid such as the tonicity, taste, temperature, etc. may influence the effect.

During dehydration, the CSF AVP levels have also been reported to increase, and return to basal levels in response to subsequent rehydration (Simmon-Opermann *et al*, 1983). ICV infusion of hypertonic NaCl and haemorrhage have also been found to elevate the CSF AVP concentrations (Szcepanska-Sadowska *et al*, 1983). Little is known about the possible role of these variations of AVP in CSF, but the observations are in agreement with the idea that the peptide may be actively secreted into the cerebroventricular space (see above).

Beside the well-documented effects of AVP in fluid balance control, this peptide may also be involved in cardiovascular control (Share, 1988). Intravascular infusion of AVP, even in amounts causing elevation of the plasma concentration within the physiological range, induces vasoconstriction, which differs in magnitude between species and vascular beds (Hock *et al*, 1984). Rather high plasma levels (>50 pmol/l) are needed to increase the blood pressure in normal animals, since the vasoconstrictive effect of AVP is effectively buffered by the baroreflex (Cowley *et al*, 1980). It has also repeatedly been demonstrated that the huge release of AVP during haemorrhage, is of importance for the maintenance of the blood pressure in this situation (Share, 1988). Less certain, however, is the possible blood pressure supporting role of AVP during dehydration (Woods and Johnston, 1983; Gregory *et al*, 1988). In addition, chronic states with elevated plasma AVP concentration such as certain forms of inappropriate secretion of AVP are usually not associated with hypertension.

2. Atrial natriuretic peptide (ANP)

Atrial natriuretic peptide belongs to a group of peptide hormones sharing common features such as natriuretic, diuretic and vasorelaxant properties. This group of peptides have similar tissue distribution of gene expression, biosynthetic pathways and pharmacological effects on target tissues (Brenner *et al*, 1990; Rozenzweig and Sedman, 1991). Based on their similarity to one of the major peptides in this group they are classified into type A, B or C. Type A is the group under which ANP is classified. ANP, also known as Atrial Nariuretic Factor (ANF), atriopeptin or A type natriuretic peptide, is the prototype of the family and is primarily produced in the atria of the heart (Brenner *et al*, 1990). Type B is usually called brain natriuretic peptide (BNP) although it is mainly produced in cardiac tissue. C-type natriuretic peptide is found primarily, or maybe exclusively, in the brain (Sudoh *et al*, 1988).

ANP is stored in dense granules in atrial myocytes as pre-ANP which has 151 amino acid residues and secreted to the circulation as ANP with 28 amino acid residues. Decades after the granules in the atria were first described by Kish in 1964, de Bold and his colleagues (1981) noticed their similarities with that found in other endocrine tissues and later described the changes in the number of these granules with salt and water intake. Some years later this group described the diuretic as well as the natriuretic property of atrial extracts which they called atrial natriuretic peptide (ANP) (de Bold *et al*, 1981).

The human ANP circulates usually as a 28 amino acid residue peptide. It has a strong structural similarity with that of rat ANP, where methionine is substituted for isoleucine at position 12 in the latter (Brenner *et al*, 1990). ANP-IR is seen

in many tissues with the highest ANP content in the atria, and it is estimated that ANP could account for 0.5-3% of the total atrial mRNA content (Hodsman *et al*, 1985). In other tissues such as the kidney, adrenals, aortic arch, lung, pituitary, the hypothalamus and the cerebral cortex, the concentration is so low that their role in contributing to the physiological plasma ANP levels is 100-fold lower than that of atrial origin. ANP levels taken from coronary sinus is found to be three times that found in plasma indicating the dominant atrial secretory site (Ledsome *et al*, 1985).

In intrauterine life and in certain clinical conditions, the ventricles have also been shown to synthesize ANP (Latton *et al*, 1986). It is the fetal ventricles that synthesize ANP and the atria take over this function some time after birth (Latton *et al*, 1986). In adults with congestive heart failure or ventricular hypertrophy, there is recruitment of the ventricles for ANP secretion (Tikkanen *et al*, 1985; Burnett *et al*, 1986).

In humans, the ANP gene is located on the short arm of chromosome 1, with three exons separated by two introns. Several receptors, which are trans-membrane proteins have been described (Almedia, *et al*, 1989; Brenner *et al*, 1990). These receptors are coupled with separate signal transduction mechanisms and result in different physiological responses. There are three major groups designated ANP-R1, ANP-R2 and ANP-R3 (Almedia *et al*, 1989). ANP-R2 is also known as clearance receptor or type C receptor and has a high affinity for ANP and helps to clear the peptide from the circulation (Sudoh *et al*, 1988; Almedia *et al*, 1989).

The most common and well established stimulus for ANP secretion is atrial stretch (Hodsman *et al*, 1985; Epstein *et al*, 1987). In rats, inflation of a balloon in the atrium results in an increase in urine flow, which could be prevented if atrial

stretch was avoided (Hodsman *et al*, 1985). This is also shown in humans during postural changes (Arjamaa *et al*, 1992) and in camels by varying the degree of right atrial stretch (Dahlborn *et al*, 1992). Atrial stretch is also shown to augment ANP secretion in dogs which develop high levels of ANP when subjected to experimental mitral valve obstruction (Ledsome *et al*, 1985).

The exact mechanism how the mechanical stimulus is transduced into increased ANP secretion is not well understood. Neither vagotomy nor beta-blockade alter the response. Calcium, which has an important electro-mechanical function in the heart is suggested as an important signalling agent. Atrial stretch may increase intracellular calcium by affecting calcium channels, and this high calcium level can induce ANP synthesis and secretion which is also the case with certain pharmacologic agents (Brenner *et al*, 1990). It has also been shown that ANP suppresses both sodium and calcium currents indicating a negative feedback mechanism for calcium mediated ANP release (Rothenzwig and Seidman, 1991).

The other stimulus for ANP secretion is an increase in atrial pacing frequency (Ellenbogen *et al*, 1988). In patients with supra-ventricular tachycardia, ANP levels are significantly elevated, and this is suggested as a cause for the frequent polyuria that these patients develop (Sagnella *et al*, 1985).

In humans and other animals salt as well as volume loading causes a rise in plasma ANP levels while water and salt deprivation decreases ANP levels (Armajaa *et al*, 1992; Dahlborn *et al*, 1992). This suggests that plasma volume and osmolality are important for the control of ANP secretion.

Besides the mechanical stimulus arising from the atria, there are a number of humeral substances that modify ANP secretion. In adrenalectomized animals

atrial stretch does not stimulate ANP secretion (Garcia *et al*, 1985) and glucocorticoids tend to permit the atria to respond to changes in atrial pressure. Administration of the mineralocorticoid, deoxycorticosterone acetate (DOCA) in adrenalectomized animals increase ANP levels by 70% in 12 hours while glucocorticoids and thyroid increase level of ANP mRNA in intact animals as well as cultured cardiocytes, via their effect on gene transcription (Garcia *et al*, 1985).

Other humeral agents like acetylcholine, epinephrine, AVP and ANG II stimulate ANP release and this seems to be via their effect on systemic blood pressure. Endothelin, which is a potent endothelium-derived constricting peptide stimulates ANP release both *in vivo* as well as *in vitro* and because of their opposing effect in various tissues, the possibility that these peptides could be biological antagonists has been suggested (Stash *et al*, 1989).

ANP is low or undetectable in healthy individuals (Tikkanen *et al*, 1985). The plasma half-life of ANP is about 2-4 min (Yandle *et al*, 1986) and is metabolized in the kidneys, liver, lungs, plasma and heart in that rank order (Tang *et al*, 1984). Endocytosis by receptors, especially type C (clearance receptor) is another way that helps to clear the peptide from the circulation (Almedia *et al*, 1989). The other method of inactivating the peptide is by breaking the central ring structure of the peptide by proteolytic enzymes in the plasma, resulting in altered receptor binding (Brenner *et al*, 1990).

Through its combined effect on the cardiovascular system, kidneys and the adrenals, ANP functions as one of the important peptides regulating hydromineral balance and blood flow (Burnett *et al*, 1984; Brenner *et al*, 1990). Infusion of ANP at physiological doses results in diuresis and natriuresis both in hypertensive as well

as normotensive rats (Morsing *et al*, 1992). This is shown to be due to the peptide's direct tubular effects to increase glomerular filtration rate and/or altering the tubulo-glomerular feedback sensitivity, which is lowered during ANP infusion (Morsing *et al*, 1992).

Infusion of ANP into hypertensive and normal volunteers leads to a sustained reduction in mean arterial blood pressure, an increase in plasma protein concentration and haematocrit levels, as well as diuretic and natriuretic effects. These effects are not merely due to the peptide's effect on the kidneys, but there is also a net loss of protein free transudate from the vascular space to the interstitium (Tikanen *et al*, 1985). There is strong evidence that this peptide has a direct effect on sodium and water transport in renal collecting ducts, but the haemodynamic alterations could also contribute to the peptide's role in fluid and electrolyte balance (Brenner *et al*, 1990).

Atrial natriuretic peptide-IR neurons and fibres are found in many areas in the brain known to be involved in central regulation of fluid and electrolyte balance. The paraventricular nucleus, which is a site for AVP synthesis receives dense innervation from ANP-IR fibres (Standaert *et al*, 1987). In one study single neurons from the PVN of anaesthetized rats were shown to be inhibited by ANP (Standaert *et al*, 1987) and ICV as well as IV infusion of ANP is also shown to suppress basal as well as dehydration or haemorrhage stimulated secretion of AVP (Samson, 1985). This suggests that ANP may modulate AVP secretion centrally.

Atrial natriuretic peptide has a vasorelaxant effect on different blood vessels taken from different regions of many mammalian species (Cody *et al*, 1986; Edwards *et al*, 1986; Saito *et al*, 1987, Rozenzwig and Seidman, 1991). The renal

vessels are primarily sensitive to ANP in this regard. ANP, by relaxing small vessels, is also shown to increase capillary permeability (Bestle and Bie, 1991) resulting in the transfer of protein-free transudate from the vascular space. The vasorelaxant property of ANP as well as BNP has been demonstrated also *in vitro* (Sudoh *et al*, 1988). In other studies ANP is shown to relax pre-constricted resistance vessels, but not those which are at their basal states (Lappe *et al*, 1985).

The sustained decline in mean arterial pressure during ANP infusion is the result of a direct effect of the peptide on both blood vessels and the heart (Breuhaus *et al*, 1985). In studies conducted in various mammals, ANP caused a decrease in stroke volume, cardiac output and heart rate (Allen and Gellai, 1987). This was shown to be due to the action of the peptide in stimulating vagal afferent and decrease sympathetic drive to the heart. In another study (Lappe *et al*, 1985) ANP resulted in a decrease in coronary blood flow and cardiac output, in isolated and perfused hearts from the rat, guinea-pig and dogs suggesting the possibility of a direct effect on the heart. It is worth noting that ANP has a vasoconstrictor effect on coronary blood vessels, opposite to its effect on other vessels.

In the kidney ANP causes increased excretion of sodium and water as well as excretion of phosphate, calcium, magnesium and chloride (Breuhaus *et al*, 1985). ANP has been shown to affect sodium and water reabsorption in the collecting ducts, but it could also alter proximal tubule transport, in particular for sodium and water (Epstein *et al*, 1987, Brenner *et al*, 1991).

Atrial natriuretic peptide, besides its direct vasorelaxant effect on blood vessels, inhibits renin secretion and release, thereby antagonizing the vaso-constrictive effect of the renin angiotensin system (Burnett *et al*, 1984; Henrich *et*

al, 1986; Henrich, 1987). ANP and the renin-angiotensin-system seem to have opposing physiological properties. It inhibits aldosterone synthesis and release, both directly as well as through inhibition of renin release. On the other hand, adrenal corticoids have a permissive and stimulatory effect in ANP secretion (Garcia *et al*, 1985; Henrich, 1987).

In congestive heart failure and liver cirrhosis, especially with ascites, there is increased secretion of ANP which is proportional to the severity of the illness. But there is decreased sensitivity to the peptide due to down-regulation of ANP receptors in these cases (Tikanen *et al*, 1985; Cody *et al*, 1986). It is also shown that ANP levels in such cases are proportional to the severity of the illness. Infusion of ANP in these states doesn't have a strong diuretic and natriuretic effect as seen in otherwise healthy individuals (Cody *et al*, 1986).

In renal diseases with nephrotic syndrome, there is a diminished level of ANP because of the intravascular volume contraction and head-up tilt experiments or head-out water immersion tests fail to stimulate ANP secretion in such conditions. Renal tubular insensitivity is suggested as a possible cause of this failure in such situations in the kidney.

3. Neuropeptide Y (NPY)

Neuropeptide Y, a 36 amino acid residue peptide, was first isolated from porcine brain by Tatemoto and co-workers (1982). It belongs to a group of peptides in the NPY/PP family and has a structural similarity with vasoactive intestinal peptide (VIP). This family of peptides is rich in tyrosine residues and is highly conserved in nature (Greenley *et al*, 1988; Sheikh *et al*, 1988). The major peptides

in this family are NPY, peptide YY (PYY) and pancreatic polypeptide (PP) (Lundberg and Tatemoto, 1982).

Immuno-cytochemical studies show NPY-like-IR in many regions of the brain, especially in those areas controlling cardiovascular functions (Peribone *et al*, 1991). NPY-IR is also observed in the arcuate nucleus, around the third ventricle and median eminence (Rudehill *et al*, 1987). In the spinal cord NPY-IR is seen in the intermedio-lateral cell columns of thoracic and sacral segments in parallel with cholinergic neurons (Chen *et al*, 1990). Peripherally NPY is stored in sympathetic nerve terminals and is co-released with noradrenaline following sympathetic stimulation (Pernow *et al*, 1986; Rudehill *et al*, 1987; Irwin *et al*, 1991; Winther *et al*, 1992).

NPY has also been found in the adrenal medulla and pancreas (Lundberg *et al*, 1986; Greenley *et al*, 1988). Soren and co-workers (1988) have compared the relative release of NPY by electric stimulation of the splenic nerve in comparison with the vagus nerve in the pig pancreas. In this study there was a release of NE as well as NPY when the splenic nerve was stimulated. However a sevenfold increase in NPY levels without altering NE levels was observed when the vagus nerve was stimulated, suggesting possible role of NPY in the parasympathetic nervous system as well.

Peptide YY is primarily found in mucosal endocrine cells of the distal ileum,colon and rectum, whereas PP is found in the endocrine pancreas (Greenley *et al*, 1988). However peptide YY has also been demonstrated in the rat medulla (Pieribone *et al*, 1991).

There are two types of NPY receptors described, namely Y1 and Y2. Pre-synaptic receptors are mainly Y2, while post-synaptic receptors could be either Y1 or Y2 (Pernow *et al*, 1986; Harfstrand *et al*, 1987). The fact that NPY is stored and released with NE and its central location in areas associated with cardiovascular control (Chen *et al*, 1990) indicate its possible role in the regulation of blood flow and maintenance of blood pressure.

Central administration of NPY causes a decrease in mean arterial pressure and heart rate by inhibiting the sympathetic nervous system. This is seen in intrathecal as well as ICV administration of the peptide (Chen *et al*, 1990). This effect of NPY can be blocked by alpha$_2$-adrenoceptor antagonists. From this and other studies it is suggested that NPY in the adrenal gland may act as an endogenous inhibitor of catecholamines, while that found in the central nervous system may be important in vasomotor centre control. NPY has an inhibitory effect on NE release at the pre-junctional level, while it potentiates the vasoconstrictor effect of NE at the neuro-effector junction (Pernow *et al*, 1986; Sheikh *et al*, 1988). Peripherally NPY is also shown to potentiate the effect of other vasoconstrictive agents besides NE (Edvinsson *et al*, 1984).

Neuropeptide Y has a vasoconstrictor property in many vascular beds as in isolated vessels from the pig splenic arterial rings and human skeletal muscle arteries (Pernow *et al*, 1989). The maximal effect of NPY in this study was seen at 500 ngm which was similar to NE and phenylephrine. Endothelium removal had no effect on its action suggesting its direct effect on vascular smooth muscles. This effect of NPY is also shown in saphenous vein from human subjects (Franco-Cereceda and Lundberg, 1987; Luu *et al*, 1992). In the above study NPY, at a

concentration of 10^{-10}-10^{-7} mol/L caused concentration-dependent constriction of resting vessels and reached its plateau after 20 min. In this study as well removal of endothelium had no significant effect on the action of the peptide.

As mentioned earlier, NPY located in sympathetic nerves supplying blood vessels plays an important part in the peptidergic control of blood flow in different vascular beds (Lundberg *et al*, 1982; Modin *et al*, 1993). This is demonstrated in the pig spiral modiolar artery involved in hearing (Carlisle *et al*, 1990) and the urogenital tract of other mammals (Edvinsson *et al*, 1984; Fahrenkrug *et al*, 1989). In this aspect NPY may act as a sympathetic neurotransmitter or neuro-modulator.

The role of NPY in the maintenance of blood pressure is shown in experimental bleeding in pigs (Rudehill *et al*, 1987). Withdrawal of 44% and 53% of blood in a step wise manner resulted in a significant release of NPY as well as NE, suggesting some role of this peptide during hypovolemic shock. Intravenous infusion of NPY in doses seen during shock resulted in a decrease in blood flow to skeletal and mesenteric beds, while those to the heart and brain were less affected. This indicates the importance of the peptide in the maintenance of blood flow to the vital organs and its regional specificity during hypovolemic states. The role of NPY in the maintenance of blood pressure during haemorrhage is also shown in other studies which showed parallel increase of NPY and NE during haemorrhage (Maisel *et al*, 1989). Whether NPY is potentiating the effect of NE or it is having a role in itself needs further studies.

Besides its effect on blood vessels this peptide has also been shown to play an important role in the control of cardiac function. It causes vasoconstriction in the coronary circulation especially in intra-myocardial arterioles (Svendsen *et al*, 1990).

In vivo as well as *in vitro* experiments show that NPY has a coronary vaso-constrictor effect and variable inotropic as well as chronotropic effects (Rigel, 1988). Application of stimuli such as capsaicin, nicotine or ischaemia are known to release neuropeptides stored in the heart. In this regard only nicotine was shown to release NPY, while capsaicin and ischaemia released sensory neuropeptides such as CGRP and SP. NPY inhibits the vasodilatation induced by metabolites such as adenosine suggesting its possible role as a neuromodulator in the regulation of coronary blood flow (Franco-Cereceda *et al*, 1987).

Neuropeptide Y is suggested to play some role in hydromineral balance. However its exact role in fluid and electrolyte balance is not well documented. In one study in humans NPY, was shown to increase jejunal fluid transport with net absorption of water and electrolytes under basal conditions and decrease the secretion of electrolytes induced by an intraluminal prostaglandin E_2 (Holzer-Petsche *et al*, 1991). This suggests that NPY could change intestinal water and electrolyte transport from secretion to absorption. This increase in the absorbtion of water, sodium and chloride occurs without an alteration in perfusion pressures suggesting a direct action of the peptide (Holzer *et al*, 1991). Centrally administered is also shown to cause gastric emptying and intestinal transit (Matsuda *et al*, 1993). NPY located in intestinal endocrine cells is also shown to inhibit gastric, pancreatic and intestinal fluid and electrolyte secretion.

The possibility that this peptide could be involved in modifying natural killer cell activity has been suggested recently (Irwin *et al*, 1991). It is known that chronic stimulation of the sympathetic nervous system results in elevated NPY levels and depressed natural killer cell activity (Irwin *et al*, 1991). The fact that NPY-IR is

demonstrated in lymphoid tissues such as the spleen suggests that the peptide could be involved in this system (Harfstrand *et al*, 1987). Splenic nerve stimulation is shown to increase both NE and NPY levels where the peptide causes an alpha adrenoceptor-independent increase in perfusion pressure and capsular contraction (Sheikh *et al*, 1988).

4. Calcitonin-gene-related peptide (CGRP)

The calcitonin gene codes for three distinct peptides namely: calcitonin, katacalcin and calcitonin-gene-related peptide (CGRP) (Ahren, 1989). The first two, primarily produced in the C-cells of the thyroid gland, are involved in calcium homeostasis, though the exact role of katacalcin is not well known. On the other hand, CGRP is mainly produced in nervous tissues and has various physiological functions. In rare conditions affecting the thyroid gland, such as thyroid carcinoma and other thyroid tumours, CGRP may be produced in the thyroid gland itself (Ahren, 1989; Steenbergh *et al*, 1984).

CGRP, first isolated and characterized by Morris and co-workers (1984), is a 37 amino acid residue neuropeptide, widely distributed in CNS as well as in peripheral nerves (Rosenfeld *et al*, 1983; Lundberg *et al*, 1985). In peripheral nerves, CGRP is especially localized in primary sensory neurons supplying blood vessels and mucous membranes (Lundberg *et al*, 1985; Hua, 1986; Brain *et al*, 1991). It is found in small, un-myelinated nerve fibres associated with blood vessels and in free nerve endings of skin (Fuller *et al*, 1987). It is stored and released with substance P (SP) in sensory nerves and the possible control of CGRP metabolism by SP is also described (Lundberg *et al*, 1985; Brain and Williams, 1988).

The cellular mechanism by which CGRP acts is not very clear. Whether CGRP is acting directly on vascular smooth muscles or via the release of nitric oxide (NO), from vascular endothelial cells may need further studies. Both direct as well as NO-dependent mechanisms are suggested (Greenberg *et al*, 1987; Persson *et al*, 1991). Recently, Samuelson and Jernbeck (1991) have suggested the possibility that CGRP could have both endothelium and non-endothelium-dependent pathways and differences in the density of CGRP receptors in various vascular beds could be the reason for the conflicting results reported on the mechanism of action of this peptide.

CGRP causes arterial dilatation and hyperpolarization of smooth muscles which is reversed by blockers of ATP-sensitive potassium channel (Nelson *et al*, 1990). Using the patch clamp technique in single smooth muscle cells, CGRP opens single potassium channels and this could explain a substantial part of the relaxation caused by the peptide (Nelson *et al*, 1990; Clausen and Andersson, 1991). Stimulation of adenylate cyclase and prostacyclin release by CGRP is also suggested for the peptide's vasodilatory action (Crossman *et al*, 1987).

CGRP has a vasodilator property in many vascular beds of different mammalian species including normal and hypertensive rats (Kawassaki, 1988; Gardner *et al*, 1989; Gardner *et al*, 1991), cats (Andersson, 1989), rabbits (Knight *et al*, 1988) pigs (Pernow, 1989; Stajarne *et al*, 1991) and human (Brain *et al*, 1985; Pernow, 1989; McDoland *et al*, 1989; Jernbeck *et al*, 1990). In lower doses intravenous infusion of CGRP causes vasodilation in the common carotid, renal, mesenteric and hind limb blood vessels, with marked effect in the carotids (Gardner

et al, 1989), showing regional differences. In higher doses CGRP lowers blood pressure and its vasodilatory effect becomes non-selective.

The vasodilator effect of CGRP is more marked in intracranial vessels. Chronic IV infusions of CGRP in rats was conducted to study the long term effects of the peptide (Gardner *et al*, 1991). In many vascular beds CGRP caused vasodilation with a decreasing potency with time but there was a marked individual variation. This and other studies indicate that CGRP could be of some clinical value in conditions, like migraine and cerebral vasospasm following subarachnoid haemorrhage (Johnston *et al*, 1990).

The effect of IV and IA infusion of CGRP on forearm blood flow and cutaneous blood flow was studied in healthy volunteers (Jernbeck *et al*, 1990). In this study infusion of CGRP at concentrations of 11-216 pmol/min in the branchial artery increased forearm blood flow which persisted for 90 minutes after the infusion was stopped. Repeated infusions had similar results. IV infusions of the peptide in doses of 104-520 pmol/min caused flush in the face, neck, upper trunk and upper arms and an increase in forearm blood flow and lowered blood pressure slightly and increased the heart rate (McDoland *et al*, 1989).

Capsaicin, a sensory stimulant, causes an increase in arterial, venous, superficial mucosal blood flow and mucosal volume due to the release of vasodilator neuropeptides such as SP and CGRP (Stjarne *et al*, 1991). Infusion of SP and CGRP also have similar responses as seen with capsaicin treatment. Numerous CGRP-IR varicose nerve fibres are also seen in the sinus node of the guinea-pig atrium, which disappear with capsaicin treatment (Franco-Cereceda *et al*, 1987).

Besides its effect on blood vessels, CGRP has a direct stimulatory effect on the heart. Intravenous administration of CGRP at a rate of 1.5 micro gm/minute caused tachycardia without lowering blood pressure in normal healthy volunteers (McDoland *et al*, 1989). This has also been demonstrated by stimulating NANC nerves in the guinea-pig heart, which has a positive chronotropic effect (Saito *et al*, 1986). Besides its positive chronotropic effect, CGRP is also shown to prolong the action potential in the pig heart (Franco-Cererceda *et al*, 1988) and the possibility that this peptide could be acting as a potential neurotransmitter in NANC nerves in the heart, with a positive chronotropic effect is suggested (Saito *et al*, 1986).

ICV and IV infusions of calcitonin and CGRP were compared with regard to hydromineral excretion in the sheep (Appelgren *et al*, 1986). In this model, unlike calcitonin , CGRP had no significant effect on renal handling of minerals. CGRP is also shown to take part in pre-syncopal symptoms that develop during head-up tilt experiments (Matzen *et al*, 1991). Tilting downwards by 50 degrees was associated with a fall in blood pressure and induced changes in blood pressure, heart rate, peripheral resistance, NE and CGRP levels. CGRP level increased and was associated with the appearance of the pre-syncopal symptoms (Yndgaar *et al*, 1991; Matzen *et al*, 1991). Levels of CGRP rise during hypovolemic as well as septic shock (Yndgaar *et al*, 1991) and this may contribute to the maintenance of perfusion to the vital organs in such circumstances.

Besides its action on the cardiovascular system, CGRP has also been shown to decrease gastric acid secretion in many animals, both basal as well as stimulated acid secretions (Pappas *et al*, 1986). This effect of CGRP is shown not to be associated with modifying gastric blood flow (Leung *et al*, 1987) and occurs when

infused IV as well as ICV (Lenz *et al*, 1985). CGRP binding to specific receptors in the pancreas is shown to decrease exocrine pancreatic secretion without altering the endocrine function of the gland in healthy volunteers (Seifert *et al*, 1985; Beglinger *et al*, 1988). CGRP-like IR has also been demonstrated in the rat pancreas (Sternium *et al*, 1986)

In other non-vascular smooth muscles, CGRP is shown to have an inhibitory effect on the oesophagus (Ratten *et al*, 1988) and vas deference. It is also found in the urogenital tract with other neuropeptides like SP and VIP and as part of the peptidergic transmitter in this region it may be involved in the control of sexual function in males (Fahrenkrug *et al*, 1989).

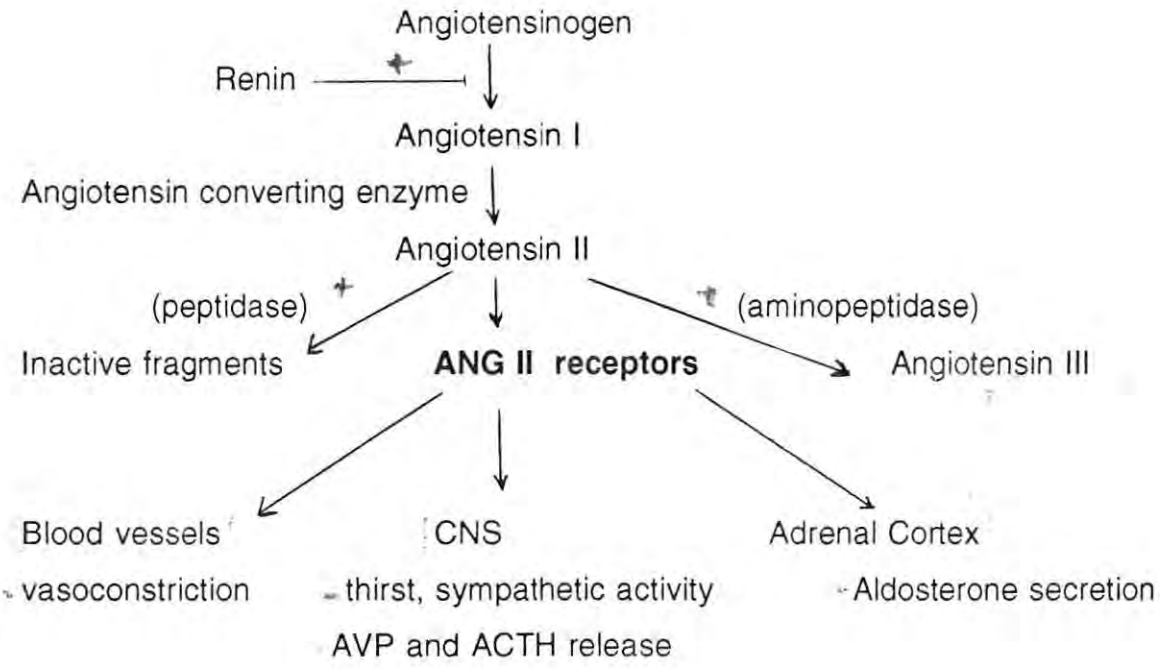
CGRP has also been shown to exert some effect on the respiratory tree and may be involved in some bronchial responses to external irritants (Lundberg *et al*, 1985; Karlson *et al*, 1991).

CGRP is found to co-exist with acetylcholine in motoneurons innervating skeletal muscles, and high density of CGRP receptors are localised at the motor end plate (Clausen and Andersson, 1991).

Because of its prolonged vasodilator property CGRP has been shown to play some role in inflammatory responses (Brain and Williams, 1989). Infusion of mediators of inflammation, such as Leukotrine B4 caused a lesser inflammatory oedema when infused alone than together with CGRP (Buckley *et al*, 1991). The synergism between CGRP and other mediators of inflammation is also claimed by others (Buckley *et al*, 1991). Involvement of CGRP in disease conditions like Reynaud's disease, inflammation of the eye, temporo-mandibular arthritis and subarachnoid haemorrhage is also documented (Brain *et al*, 1990; Johnston *et al*, 1990, Appelgren *et al*, 1991).

5. Angiotensin II (ANG II)

The initial observation on the biological effects of the renin-angiotensin-system (RAS) were made at the end of last century. The long-lasting blood pressure increase seen after injection of crude renal extracts described by Tigerstedt and Bergman in 1898 was interpreted to be due to a pressor agent which they named renin. Renin was later demonstrated to be a proteolytic enzyme which acts on a substrate in plasma leading to the formation of a pressor substance known as angiotensin II (Page and Helmer, 1940). Intense studies of the RAS during the last decades have identified the biochemical pathways of the system, its distribution in different tissues, and several of its physiological and pathophysiological effects. More recently, the mode of action and the molecular biology of the system has been elucidated. The biochemical cascade of the RAS and the major biological effects of ANG II can be summarized as in the figure below.



Renin secretion, stimulated by many factors, is the rate limiting step in angiotensin formation (Ben-Ari and Garrison, 1988).

A wide range of function has been ascribed to Angiotensin II, both peripherally as well as centrally. Peripherally circulating ANG II stimulates the release of aldosterone, causes systemic vasoconstriction and acts on sympathetic nerve terminals to release neurotransmitters, while centrally ANG II stimulates AVP secretion and induce thirst (Lind *et al*, 1985). ANG II is acting both in the CNS and peripheral target tissues in regulating fluid and electrolyte balance as well as blood pressure (Andersson *et al*, 1978).

Based on sensitivity to antagonists and response to reducing agents, two types of receptors namely ANG II AT-1 and ANG II AT-2 are described. The AT-1 receptor is present in areas involved in the functions of ANG II in blood pressure control and fluid homeostasis, whereas ANG II binding in areas not related to these functions is due to ANG II AT-2 receptor subtype (Tsutsumi and Saavedra, 1991). AT-1 receptors are found more frequently in adult animals, while AT-2 receptors are more abundant in young animals. These differences in the densities of receptors during development, have indicated the possibility of different functions of these receptors. AT-2 receptors are found in areas involved in the control of learning of motor activity, sensory areas and limbic structures while ANG II AT-1 receptors are related with other known functions of ANG II (Tsutsumi and Saavedra, 1991). ANG II receptors have been shown to play a role in salt intake as early as the first and second week after birth (Tsutsumi and Saavedra, 1991).

A number of factors are shown to modulate the density of ANG II receptors. In a study in rats these receptors were shown to be down-regulated by low salt

intake (Sahlgren *et al*, 1992) and this was modulated by sympathetic nervous system and AVP. In the above study arginine vasopressin blockade doubled ANG II receptor densities while renal denervation or guanethidine treatment increased the renal ANG II receptors by more than 50%. In renal hypertension ANG II receptor density increases which is shown to be independent of changes in blood pressure.

Centrally ANG II reactive neurons and fibres are localized in those areas controlling fluid and electrolyte balance especially at the subfornical organs (Lind *et al*, 1985 and Saavedra *et al*, 1986). Water deprivation increases ANG II-IR in these areas, while nephrectomy and water loading are associated with the opposite effect. In a study in rats, high sodium balance was shown to inhibit the RAS while low sodium levels had the opposite effect (Iwao *et al*, 1988).

ANG II, infused centrally as well as peripherally stimulates drinking. This is by binding to specific receptors in the circumventricular organs which are accessible from both sides of the blood brain barrier (Saavedra *et al*, 1986). ICV infusion of the peptide has a longer and stronger effect in smaller doses compared to peripherally administered ANG II. This is suggested to be due to the slower degradation rate of the peptide in cerebrospinal fluid than in plasma (Simon-Opperman *et al*, 1987).

Hypertonic sodium chloride infused ICV or peripherally is also shown to induce thirst and result in drinking as that observed with ANG II infusion (Andersson, 1978) and this had led to the notion that ANG II and hypertonic saline could be detected by the same type of sensors. However the existence of separate sensory sites for these two stimuli was latter demonstrated (Eriksson *et al*, 1988). The relative responses of neurons taken from the SFO, OVLT and other circum-ventricular organs taken from the duck brain showed that maximal stimulatory effect

on SFO neurons were caused by ANG II, while hypertonic saline had minimal effect. In neurons taken from the OVLT area hypertonic saline had maximal effect while ANG II had minimal effects. Destruction of the SFO causes minimal effects on osmotically-induced thirst but resulted in diminished response to angiotensin II stimulated drinking (Thrasher and Simon, 1984). The SFO, with high densities of ANG II receptors, seems to be the most likely site for the peptide's action.

ANG II, besides inducing thirst, also stimulates AVP secretion administered centrally as well as peripherally (Ramsay *et al*, 1978). This effect of ANG II has been shown to be due to its vasoconstrictive effect in the highly vascularized regions of the circumventricular organs responsible for these responses, and is blocked by vasoleptics infused centrally (Saavedra *et al*, 1986). However a direct stimulatory effect of ANG II on AVP secretion is also described and ANG II reactive fibres are also shown projecting from the SFO to the PVN, which is one of the major sites for AVP synthesis (Ramsay *et al*, 1978; Lind *et al*, 1985).

Besides its indirect effect in fluid and electrolyte balance, ANG II has also been shown to be involved the development of salt appetite (Blair-West *et al*, 1988) early in life. ANG II stimulates aldosterone secretion which helps retain sodium in the kidney. The intra-renal production of ANG II, causing renal vaso-constriction contribute to salt retention directly. However, this peptide has also been described as having a natriuretic property in animals deprived of water and salt (Brown and Venuto, 1991).

Angiotensin II is one of the strong vasoconstrictor peptides in the mammalian body having a direct effect on vascular smooth muscles. Its vasoconstrictive effect is well marked, especially in the kidneys (Brown and Venuto, 1991), but other

vascular beds such as cerebral and mesenteric vessels are also affected. The role of ANG II in maintaining blood pressure is shown in many studies during various degrees of hypovolemic states and ANG II and plasma renin activity are found to increase in different degrees of haemorrhage (Ullman *et al*, 1992).

In some clinical studies cases of hypertension have been associated to the action of ANG II to cause vascular contraction and/or result in increased retention of salt. In a study conducted in the sheep, infusion of captopril, an angiotensin converting enzyme inhibitor, was shown not to significantly affect the volume of blood required to induce hypotension (Ullman *et al*, 1992). The combined effect of AVP and ANG II blockade had the maximum effect in making the animals least tolerant to bleeding-induced hypotension. In the above study, ANG II was useful in the recovery of blood pressure after bleeding. In humans ANG II is shown to increase before there is a decrease in arterial pressure induced by foot down tilt or lower body negative pressure studies suggesting the peptide's role in maintaining blood pressure (Davis *et al*, 1976).

6. Substance P (SP)

Substance P was discovered in 1931, when von Euler and Gaddum were looking for acetylcholine distribution in the equine brain (Maggio, 1988). This substance has been studied extensively and its hypotensive, spasmogenic and secretagogue property described in many animal species (Pernow, 1983). Though SP was known for a long time, the final isolation and characterization of the peptide was achieved in the early 70's (Chang *et al*, 1971) trying to isolate corticotrophin releasing factor in the equine brain.

Substance P belongs to a group of peptides known as tachykinins which share common structural and chemical properties (Maggio, 1988). These peptides have a similar carboxyl terminal differing in only one amino acid residue at the forth position starting from the carboxyl end. Depending on the nature of this amino acid at this position the tachykinins are divided into two major groups: aliphatic and aromatic tachykinins. SP having phenylalanine at this position is an aromatic while the other major tachykinins, neurokinin A and neurokinin B are aliphatic (Regoli *et al*, 1990).

Substance P interacts with its receptors, neurokinin-I (NK-I) located on endothelial cells and release nitric oxide (NO) and possibly other bioactive substances for example in nerve terminals where SP stimulates the release of neurotransmitters (Regoli *et al*, 1990).

The peptide is widely distributed in the body of many animal species ranging from lower invertebrates to higher mammals. It is found in the mammalian brain, more in grey matter than in white matter with the highest binding in *substantia nigra*, where it is thought to play a role in nociception (Pernow, 1983). SP is also seen in the spinal cord, more in the dorsal roots than in the ventral. In peripheral nerves, it is found in primary sensory neurons together with other sensory neuropeptides (Lundberg *et al*, 1985) and is intimately associated with blood vessels, mucous membranes and glands (Pernow, 1983). It is widely distributed in the gastrointestinal system of frogs, fishes, and higher mammals where it exerts a strong effect on gut motility and secretion. The peptide is found in all layers of the intestine especially in the *muscularis mucosa* but is strictly confined to nervous structures (Pernow, 1983). In Hirschsprung's disease a low SP concentration is

found in the distal aganglionic segment of recto-sigmoid colon, while the proximal part shows higher concentrations.

Substance P is degraded in many tissues such as the kidney, spleen, liver and intestine. Liver and the intestines are shown to have the highest degradation capacity. Peptidases, both free as well as membrane-bound, degrade this peptide.

Substance P has various physiological effects. It stimulates intestinal smooth muscles, the isolated uterus, ureter and urinary bladder and this seems to be a direct effect on smooth muscle. In vascular smooth muscles SP causes a dose-related, vasorelaxation and this has been shown to be due to release of nitric oxide or other endogenous vasodilators (Andersson, 1987).

Substance P has been described in nociception, and is shown to stimulate neurons in *substnatia nigra* and crude extracts of SP were shown to have a sedative effect in mice and also to antagonize morphine. ICV as well as IV administration of SP causes release of growth hormone, prolactin and inhibits gonadotrophin-releasing hormone suggesting its possible involvement in adenohypophyseal secretion.

The fact that SP is distributed along the cardiovascular control pathways such as the carotid body, carotid sinus nerve, baro- and chemoreceptor pathways and in *nucleus tractus solitarius* (NTS), studies have indicated its involvement in the control of this system. SP, administered IA as well as IV causes vasodilatation and a decrease in peripheral resistance in a dose dependent manner. In man, IA infusion of SP causes a rapid and transient vasodilatation, while it causes flush in face, hypotension and tachycardia when infused IV (Pernow *et al*, 1989).

Substance P-IR is seen in different vascular beds supplied with sensory nerve fibres. It is observed in blood vessels of the skin, dental pulp, thoraco-bronchial tree, gastrointestinal tract and brain (Fuller *et al*, 1987; Prabhakar *et al*, 1987). It is mainly found in the adventitia and media of blood vessels especially arterioles. In the pig and human heart, besides its involvement with blood vessels it is also seen along the conduction system and cardiac ganglion cells.

Injection of SP in the skin, skeletal muscle and small intestinal vessels have stronger effect than seen in the carotids, hepatic, mesenteric and portal beds. Renal and cerebral vascular beds seem to be less sensitive or resistant to IV SP. This effect of SP is also shown in isolated vessels (Luu *et al*, 1992).

In humans, injection of SP at a rate 0.7 pmol/min in the brachial artery caused an increase in forearm blood flow, associated with wheal, flare, and itching responses. The above effect of SP was shown to be due to the release of histamine from mast cells (Fuller *et al*, 1987). Substance P is also shown to play a major role in antidromic vasodilation (Andersson, 1987; Pernow, 1983).

Though the peripheral infusion of substance P causes a drop in blood pressure, the central administration of this peptide is associated with a rise in blood pressure and a decrease in heart rate. Besides its effect on blood flow, SP has a diuretic as well as a natriuretic property in many animal species. In one study in dogs 0.4 pmol/kg/min or higher doses into the renal artery resulted in a dose related increase in renal blood flow, urine volume, sodium and potassium excretion and a decrease in urine osmolality while the glomerular filtration rate was unchanged. Infusion of 0.05 pmol/min in the aorta above the renal artery had a similar effect as in the dogs. Both intra-renal as well as central mechanisms are

suggested in relation to this effect. These studies show that substance P could have some role in hydro-mineral balance.

Substance P has also been shown to stimulate the release of AVP when administered IV while no significant effect is seen when administered IA (Pernow, 1983). In one study the relative bindings of SP and NKA (neurokinin A) in the hypothalamus around the magnocellular portion increases in rats given hypertonic salt solutions instead of tap water for 12 days in rats (Larsen *et al*, 1992).

C. Neuropeptides During Dehydration

When man or animals are dehydrated, water deprived or subjected to increased extracellular Na^+ concentration, there will be a corresponding rise in osmolality, which will trigger neuro-hormonal responses to counterbalance the disturbance. Correction of water deficit by drinking, minimizing further water loss by AVP, and prevention of the detrimental effects of hyperosmolality by natriuresis will occur (MacKinley *et al*, 1983; Wade *et al*, 1983). A rise in osmolality together with the reduction in ECF volume will interact to cause AVP secretion and thirst (Andersson, 1978; Bie, 1980; Szczepaska-Sadowska *et al*, 1983).

One of the main causes of dehydration is diarrhoea. There are certain indicators of the degree of severity of diarrhoea. In a recent study factors such as watery consistency of the stool, duration of more than three days, high stool rate, frequent vomiting, associated fever and protein-energy malnutrition were found to be firm indicators of severity (Lulseged, 1992). Protein energy malnutrition (PEM) is associated with prolonged diarrhoea, higher stool frequency and vomiting as well as marked dehydration (Hanssen *et al*, 1989).

Hypernatremic dehydration (serum Na^+ concentrations above 150 mmol/L) is less common but more serious than either hypo- or isonatremic dehydration (Conley, 1990; Fayday *et al*, 1992). If the sodium level is above 165 mmol/L as in the case of severe hypernatremia it is associated with higher morbidity and mortality related to CNS dysfunction (Conley, 1990). Hypernatremic dehydration is positively correlated with the severity of the diarrhoea, with increased oral rehydration solution (ORS) administration and is more common well-nourished cases.

In experimental dehydration studies, mixing errors in ORS preparations was found to be the commonest cause of hypernatremic dehydration (Prigle and Berthiaume, 1988). In one episode hypernatremic dehydration was also documented in a breast-fed child of inadequate breast milk, which was hypertonic (Thullen, 1988).

There has been a lot of discussion as to the concentration of the ORS to be given especially in hypernatremic dehydration. Many studies suggest the use of ORS 90, recommended by WHO, even in hypernatremic cases rather than using solutions of lesser concentrations. Some suggest ORS 90 as having an advantage over lesser concentrated ORS solutions, since it was quite effective in treating the dehydration as well as correcting the hypernatremia slowly preventing the risk of sudden hyponatremic convulsions. ORS 90 is also suggested for the hyponatremic patients (Hernandez *et al*, 1990). The other point in support for ORS 90 is that when the ORS sodium is higher the percentage of the sodium unabsorbed will be higher, reducing the risk of hypernatremia compared to ORS 60, and ORS 90 was associated with a better rehydration (Marin *et al*, 1988). But other reports favour the use of lower osmolality ORS since it was found to be associated with lesser

treatment failure as gauged by high stool output, persistent vomiting and ileus, and there was no difference in sodium and potassium levels achieved in plasma with the use of different ORS preparations (Velasquez-Jones *et al*, 1990).

There are behavioral, neuro-endocrine, and natriuretic responses that occur when an animal is dehydrated (MacKinley *et al*, 1983, Wade *et al*, 1983). Stimulation of thirst as well as the neuro-endocrine response during dehydration is complex and a number of hormones seem to be involved. This is illustrated in a study in newborn calves (Safwate *et al*, 1991) where plasma renin, aldosterone and AVP levels were 10-15 times higher in diarrhoeic than control calves. The high AVP levels could explain the elevated urea levels usually found in dehydrated animals.

The brain has a special adaptive mechanism in dehydration especially hypertonic dehydration. Taurine, an organic osmolyte in the brain with an osmo-protective property seems to be the most important molecule for this purpose (Trachtman *et al*, 1992). This amino acid constitutes about 50% of the adaptable intracellular osmolal pool the concentration of which varies in the course of osmo-regulation (Trachtman *et al*, 1988). Its transport is enhanced by hyperosmolality but 96 hrs water deprivation may not affect its transport significantly (Conley, 1990). Adminstration of taurine analogues affords cerebral osmoprotection during chronic hypernatremic dehydration. In the cat taurine has been shown to lessen mortality, neurological morbidity and brain cell dehydration during such dehydration states (Trachtman *et al*, 1988). It is shown that the cerebral response to chronic hypertonic stress accelerated trans-membrane flux of osmoprotective solutes in addition to mobilization from sequestered intracellular sites in an attempt to increase the cytosolic pool of osmotically active molecules. Taurine depletion was not associated

with increased risk of hypernatremic dehydration *per se*, but was associated with higher mortality, seizure activity compared to taurine replete animals (kittens), rendered vulnerable to chronic hypernatremic dehydration. In addition there was a significant decrease in brain cell water from intracellular compartment correlated well with cerebral taurine content (Trachtman *et al*, 1988).

Besides the behavioral, hormonal and cerebral responses there will also be a net excretion of sodium, natriuresis, the degree of which parallels the degree of dehydration (MacKinley *et al*, 1983; Lee *et al*, 1991; Sjoqvist *et al*, 1991). The natriuresis is homeostatic and ameliorates the rise in plasma osmolality. During rehydration following dehydration, there will be retention of sodium to counter-balance the deficit that occurred during the dehydration phase. Though the occurrence of natriuresis in various clinical conditions is known the exact control mechanism is not well understood.

Mckinley, and coworkers (1983) have suggested a central origin, especially the involvement of the OVLT. This had earlier been suggested by Andersson and his group (1978) which showed the changes in natriuresis by altering CSF sodium concentration. ICV infusion of hypertonic saline is associated with high sodium excretion while the reverse is also true. The role of the OVLT area in this aspect is also shown in ablation experiments which resulted in a general decline of osmotically induced thirst, AVP release and natriuresis. But AVP release secondary to non-osmotic stimulation such as haemorrhage was not reduced nor the natriuretic response to isotonic expansion of extracellular fluid volume. In addition, animals with OVLT lesions show an increase in AVP levels but fail to develop natriuresis during dehydration (Ramsay *et al*, 1988).

A number of experiments have attempted to explain the dehydration-induced natriuresis, but so far there are no conclusive results. AVP which has a natriuretic property in large doses, is proposed as one candidate. The role of AVP and the relative importance of the V1 and V2 receptors in this aspect was analyzed. It was found that V1 antagonists lowered the natriuresis by 50% but V2 antagonists were without effect (Lee *et al*, 1991; Sjoqvist *et al*, 1991). ANP and aldosterone were also proposed as a cause for dehydration-induced natriuresis. However there was no decrease in the concentration of aldosterone during dehydration nor there was a rise in ANP level (Metzler et al, 1986; Cowley *et al*, 1988).

ANG II is another neuropeptide suggested to be involved during dehydration. It causes thirst, AVP secretion and natriuresis when administered ICV (Andersson, 1978; Saavedra *et al*, 1986). If administered for a long time, ANG II can cause hyponatremia and hypotonicity even when the animals are deprived of water however there is no firm evidence that ANG II could be the cause for the natriuresis.

In addition to the central mechanism discussed above, the integrity of renal nerves and the intrinsic property of the kidneys may play a role in dehydration induced natriuresis. Renal nerves which are exclusively adrenergic cause anti-natriuresis when stimulated at low frequency. When the mean arterial pressure increases there will be a reflex withdrawal of renal efferent sympathetic nerve activity which will result in natriuresis and diuresis. Though the decrease in sympathetic tone is associated with natriuresis and diuresis in response to volume expansion, this response is also seen in denervated and isolated kidneys and this

may be an intrinsic property of the kidneys rather than the renal nerves (Rudenstam *et al*, 1992).

The importance of perfusion pressure to induce pressure natriuresis is described (Hall *et al*, 1988). Theses studies indicate that an increase in the renal perfusion pressure by few milimiters of mercury could result in several fold increase in sodium excretion. During dehydration a slight increase in mean arterial pressure is described and this may result in the increased natriuresis observed.

Even though many physical, neural and hormonal factors are proposed the exact mechanism of dehydration-induced natriuresis remains to be proved.

CHAPTER II

GENERAL AIMS OF THE STUDY

This study was conducted to describe further the modulating role of NO in peripheral blood flow control and its possible involvement as a neuro-regulatory agent in the CNS in hydromineral balance.

Competitive NOS antagonist has been applied in one study in the conscious sheep to elucidate the importance of NO in the central regulation of AVP secretion and fluid balance. In addition the modulating role of NO in peripheral blood flow control was investigated in rabbits.

The changes in neuropeptide levels during water deprivation and rehydration was analyzed in the sheep and serial plasma, CSF and urine levels of neuropeptides were measured and compared with plasma osmolality and electrolyte levels to study the involvement of neuropeptides in this respect.

In the last study, the electrolyte and osmolality changes during dehydration secondary to moderate to severe diarrhoea was conducted in children, to describe and compare with the changes that occur during dehydration secondary to water deprivation.

CHAPTER III

MATERIALS AND METHODS

STUDY 1 Hind Limb Blood Flow Studies in the Rabbit.

The peripheral effects of L- NAME, D- NAME, SP, and adenosine (ADO) on blood flow in relation to NO were studied using rabbits of either sex (New Zealand White species) ranging in body weight from 2.0-3.5 kg. The rabbits were kept in cages, fed cabbage, hay and cereals once a day and had free access to water with equal exposure to day and night. They were anaesthetized by sodium pentobarbital (mebumol) (6 mg/ml, 30-40 mg/kg) via an ear vein for induction of anaesthesia which was maintained by a continuous infusion solution with anaesthesia at a rate of 5 ml/kg/hr via one of the jugular veins. After securing tracheostomy the animals were allowed to breath spontaneously or ventilated with a respirator at a rate of 40/min and tidal volume of 6-8 ml/kg. Heparinized (500 IU/ml) catheters were inserted in the right carotid artery and left jugular vein for continuous recordings of systemic blood pressure and administration of peptides and drugs, respectively. Additional infusion solution which contained (D-glucose 3.6 gm/100 ml, sodium bicarbonate 0.7 gm/100 ml and 6 ml of 10% diluted sodium pentobarbital) was administered at a rate of 5 ml/kg/hour.

A longitudinal incision in the skin along the saphenous vein was made in the hind limb area. The skin was reflected and the underlying tissue dissected to expose the femoral artery on which an ultrasonic flow probe (3R 505) was applied. The probe was connected to an ultrasonic blood flow meter (T202 2-channel, Transonic System Inc) to measure the blood flow.

Another incision was made caudal to the hip joint to expose the sciatic nerve bundle. The nerve was crushed to prevent nerve-induced impulses from having central effects. Distal to the crush shielded platinum electrodes were applied and connected to a stimulator (Grass S44) via a stimulus isolation unit. Stimulation pulses (0.3-10 Hz, 0.01-3 ms, 0.1-6 V, 20-40 pulses at 5 minutes intervals) were monitored on an oscilloscope (Tektronix S103N) and timing was achieved by an electronic counter (Kessler-Ellis Products, Atlantic Highlands, New Jersey, USA).

Vascular resistance in the hind limb was calculated from the expression mean arterial pressure / femoral blood flow which was expressed in $cmH_2O/ml/min$, which was designated as hind limb resistance units (RU_H). Guanethidine (3 mg/Kg) followed by glucose/dextran (10-15 ml/kg) were administered intravenously one hour before experimental recordings. Pancuronium (0.3 mg/kg/hr) was administered intravenously. During the experiment infusion solution composed of D-glucose 3.6 gm/100 ml, sodium bicarbonate 0.7 gm /100 ml and 6 ml of 10% diluted sodium pentobarbital was administered IV at a rate of 5 ml/kg/h, by means of a syringe infusion pump (STC-521, Terumo Corp., Tokyo, Japan).

For local infusion of the peptides and drugs, a longitudinal incision was made along the opposite thigh region to expose the femoral artery and infusion was made by an IA catheter in the femoral artery to reach the abdominal aorta near the bifurcation of the common ileac artery so as to deliver the infused substance to the opposite femoral artery. Intra-arterial infusions of SP (10^{-6} 100 µl/min) and ADO (2.5 µl/min) was assessed after administration of L-NAME (10 mg/kg) IV. Baseline values were recorded in all experiments. Blood pressure, heart rate, blood flow and resistance changes were monitored throughout the experimental period.

STUDY 2 Intra-cerebroventricular Infusion Studies in the Sheep.

Sheep of Texel breed, ranging in body weight from 25-50 kgs were used. They were allowed to habituate in the experimental area for at least two weeks before any experimental procedures and were exposed to equal lengths of day and night. They were examined and dewormed after consulting a veterinary doctor.

Before any major operation, they were given sedatives plegicil IV slowly about 30 minutes before the anaesthesia. Induction of anaesthesia was achieved by 10 $mg.kg^{-1}$ sodium thiopental (10% diluted). Muscle relaxant (atropine), 1 ml IV was given just before intubation and were mechanically ventilated until spontaneous breathing was achieved. Anaesthesia was maintained by a gas mixture of oxygen, nitrous oxide and 2% enflurane.

OPERATIONS

Prophylactic antibiotic treatment was given for four days (benzyl penicillin procaine 20 $mg.kg^{-1}$, streptocilline vet., Novo, Denmark)

Carotid loop operation

When animals were ready for operation either carotid loop preparation or implantation of cannulae in the lateral ventricles were performed on the same animal. A minimum of ten days interval was given between these two operations.

The animal was placed in the supine position. Then the carotid arteries were exteriorised into cervical skin loops by two midline incisions on the skin of the neck, which was first infiltrated by local anaesthetics (xylocain-adrenalin., Asha, Sweden). The underlying tissue was teased and the arteries were dissected free of any tissue. The arteries were stripped of any nervous tissue to avoid pain in subsequent procedures. Then one or both of the carotid arteries were rapped by the overlying skin and then sutured inside (Figs.3.2. a & b).

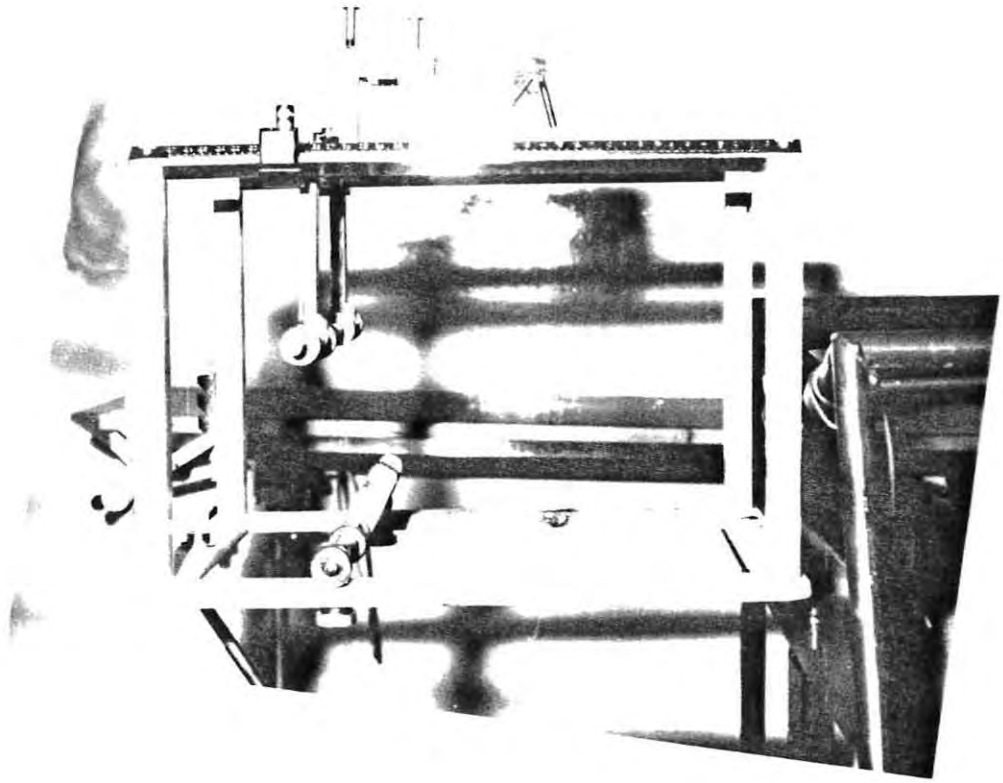


Fig. 3.1.a. Stereotaxic instrument for the sheep, lateral view.

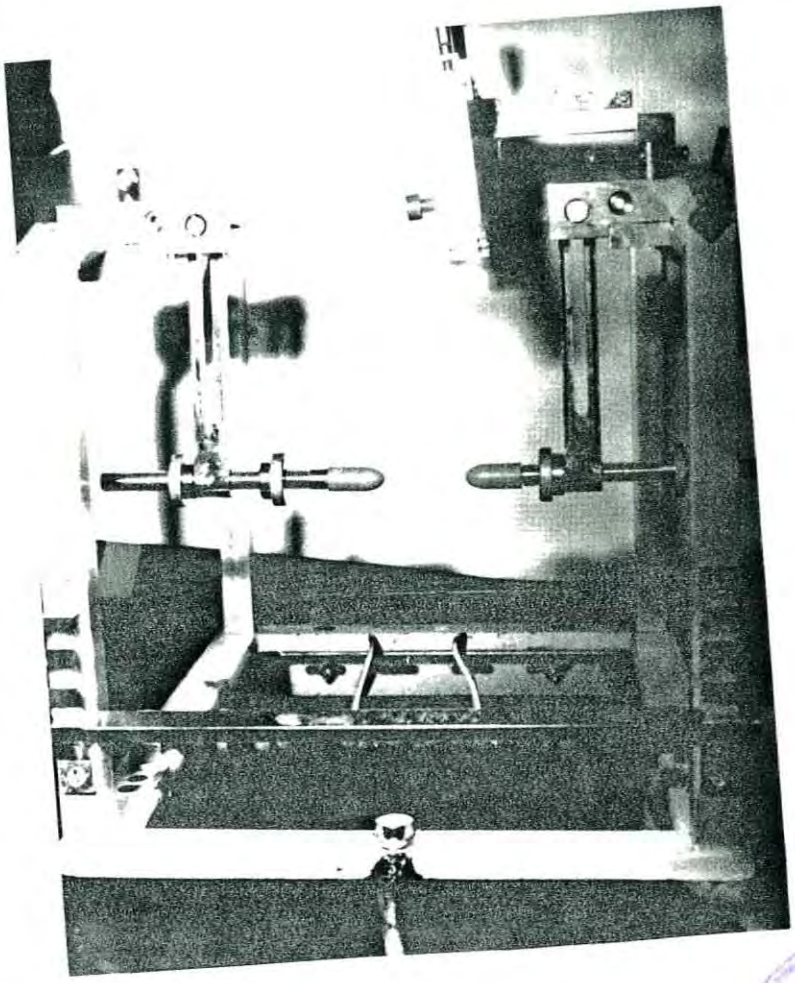


Fig.3.1. b. Stereotaxic instrument fpr the sheep, frontal view.

Fig. 3.2. a Carotid loop operation. (blunt dissection for the artery).

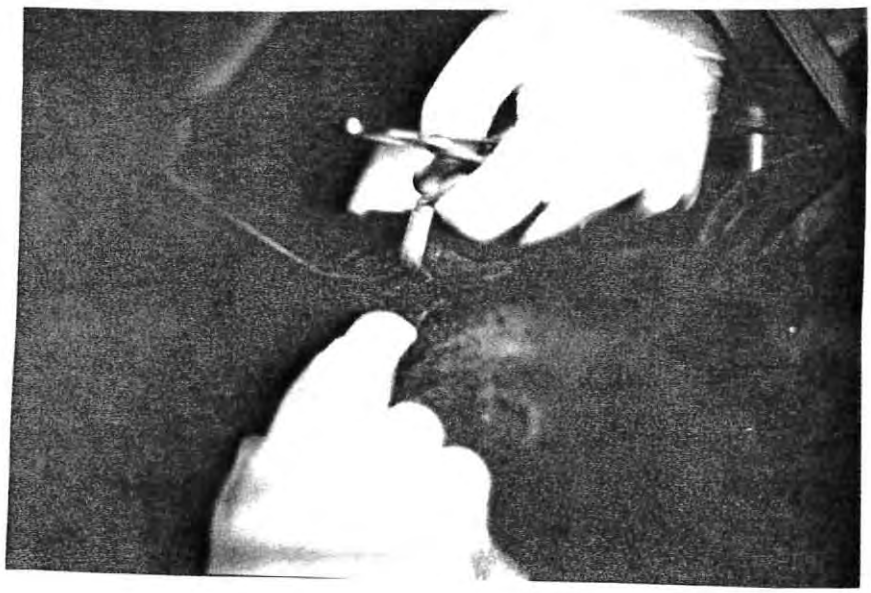


Fig. 3.2.b Carotid loop operation.

ICV cannula implantation

Operations on the lateral ventricles were performed under the same anaesthetic conditions as the carotid loop operations. The animal was placed in a standing position and the head was fixed by the guide of a stereotaxic instrument manufactured at the Department of Physiology, Karolinska Institutet, Sweden (Fig 3.1. a and b). The skin above the ventricles were infiltrated with local anaesthetics (xylocain-adrenalin., Asha, Sweden). Then a circular skin flap was dissected away to expose the skull at the junction of the cervical sutures. The periosteum was rubbed free of any soft tissue. Four holes were drilled through the skull with a dental burr around the sagittal suture for fixing the tubes in the ventricles by four stainless screws. Above the lateral ventricles two holes were made by a driller for the guide tubes through the skull.

Guided by the stereotaxic instrument stainless steel guide tubes connected to an infusion pump were lowered slowly into the ventricles aided by continuous pressure monitoring from a slowly perfused inner needle reaching 5 mm beyond the guide tube connected to a Grass polygraph. A sudden drop in the pressure recorded by the Grass polygraph will occur when the cannula enters the ventricles. When freely flowing cerebrospinal fluid was observed through the plastic tubing connected to the inner needle, the cannulae were closed by metallic blockers which could be removed when ever wanted. The cannulae were fixed to the skull by dental cement applied around the exposed part of the skull (Figs. 3.3.a & b). After each procedure the animals received four-five days intramuscular injections of antibiotics described above. The animals were observed for 2-3 weeks before any experiment was carried on.

Fig. 3.3.a ICV cannula implantation (application of dental cement).

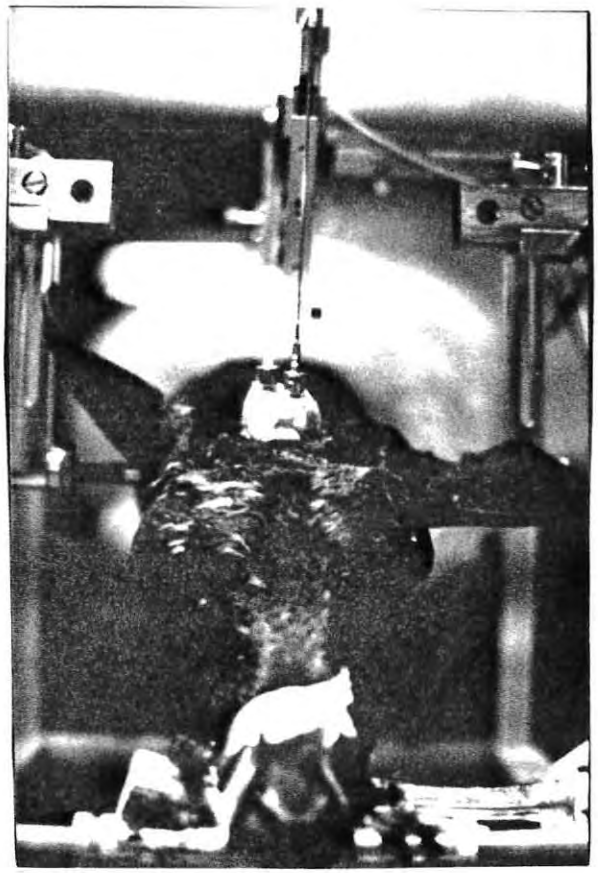


Fig. 3.3.b ICV cannula implantation (closer view).

Five sheep with ventricular cannulae and carotid loops were used to assess the influence of NO-synthase inhibitor on water metabolism. L-NMMA was infused by the aid of an infusion pump at a concentration of 10^{-3} M, 20 mico L/min, for sixty min. Blood pressure and heart rate were monitored starting 30 minutes before infusion and throughout the experiments. Urine was collected every 10 min via a Foly catheter introduced in the bladder for later determination of osmolality and of free water clearance. For comparative purposes D-NMMA (an enantiomer of L-NMMA) was also infused in the same concentration for similar periods.

Study 3 Water Deprivation and Rehydration Studies in the Sheep.

Sheep of Texel breed were prepared with carotid loops and intra-cerebroventricular cannulae in the same way as described in experimental procedures in study 2.

During the experimental period all animals were kept in metabolic cages, fed hay once a day and had free access to salt and water. In the first set of experiments four sheep previously prepared with ventricular cannulae for chronic CSF sampling and venous catheter for blood sampling were introduced. Animals were deprived of water for 96 hours and then rehydrated by getting free access to water and subsequently drinking *ad libitum*.

During the experiments, serial CSF and plasma samples were collected for analysis of osmolality as well as later RIA determinations of CGRP, AVP, ANG II and NPY levels. Blood pressure and heart rate were monitored throughout the experimental period.

STUDY 4 Studies in Dehydrated Children Secondary to Diarrhoea.

A study on dehydrated children secondary to acute diarrhoea was conducted to study the involvement of neuropeptides during such states. For this study thirteen cases with moderate to severe dehydration were selected from Ethio-Swedish Paediatric Clinic in Addis Ababa. Selection of cases was done randomly and level of dehydration was assessed based on clinical manifestations based on WHO classification of dehydration secondary to diarrhoea. Those children with other associated illnesses and frequent vomiting were excluded from the study. With the informed consent of the parents or the care taker, 2-4 ml of blood was taken from the dehydrated child just before the start of ORS therapy and another blood sample was collected after full hydration which was assessed clinically and by the increment in body weight of the child during ORS treatment. Blood samples were taken from brachial vein in most instances but on some occasions it had to be taken from the femoral vein. Samples were taken in prechilled heparinized vacutainer tubes and were centrifuged at 3000 rpm, at 4°C. Plasma was kept at -20°C till analysis was made.

Plasma sodium and potassium were measured by an AVL electrolyte analyzer (model 984-S) and total plasma protein level was estimated by a hand refractometer. Plasma osmolality was measured by an Advanced Digimatic Osmometer (model 3D2). Plasma was stored at -70 °C for analysis of neuropeptides at the two stages by radio-immunoassay.

About the same amount of blood was withdrawn from two healthy children in a similar age group. Samples were processed in the same and analyzed for similar variables for comparative purposes.

Statistics.

Results are expressed as mean ± standard error of mean (SEM). Statistical significance was evaluated using Student t-test for unpaired observations. Number of experiments or animals used is given in brackets. (NB * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$)

Neuropeptide analysis.

The concentration of AVP was analyzed in EDTA-plasma by a RIA method according to Lishajko, 1983 with a slight modification i.e. instead of separation on a Sephadex G-50 column, two ml of plasma was extracted with cold acetone and petroleum benzine. The limit of detection was 1.3 pmol/L. Plasma for ANG II determination was taken from blood collected in tubes with a cocktail of EDTA (0.125 mol/L), phenantroline (25 mol/L and neomycin (0.2%). After the same extraction procedure as for AVP analysis, ANG II was measured by radio-immunoassay described by Gray and Simon, 1985. ANG II antiserum was kindly provided by Dr. Simon, W.G. Kerckhoff Institute, Bad Nauheim, Germany. For analysis of neuropeptide Y, and calcitonin-gene related peptide analysis, samples were filtered using a reverse-phase C18 cartridge (Sep Pak, Waters) and analyzed using radio-immunoassays as described by Theodorosson-Norheim and collaborators (1987). The sensitivity for CGRP was 16 pmol/L and NPY 15 pmol/L.

CHAPTER IV

RESULTS

Study 1 Hind Limb Blood Flow Studies in the Rabbit.

The effects of sciatic nerve stimulation and infusion of substance P and adenosine on hind limb blood flow is shown in the rabbit experiments. Basal hind limb vascular resistance remained unchanged over several hours. In animals having received pancuronium (0.3 mg/kg) electrical stimulation of the sciatic nerve (2 Hz, 1 ms, 3 V, 40 pulses at 5 min intervals) in the absence of guanethidine induced transient increases in hind limb vascular resistance from 5.8 +/- 0.5 to 9.3 +/- 1.0 RU_H ($P<0.001$, n=12) (Fig. 4.1.1). In the presence of guanethidine (3 mg/kg i.v.) nerve stimulation induced a transient increase in hind limb blood flow (Fig. 4.1.2).

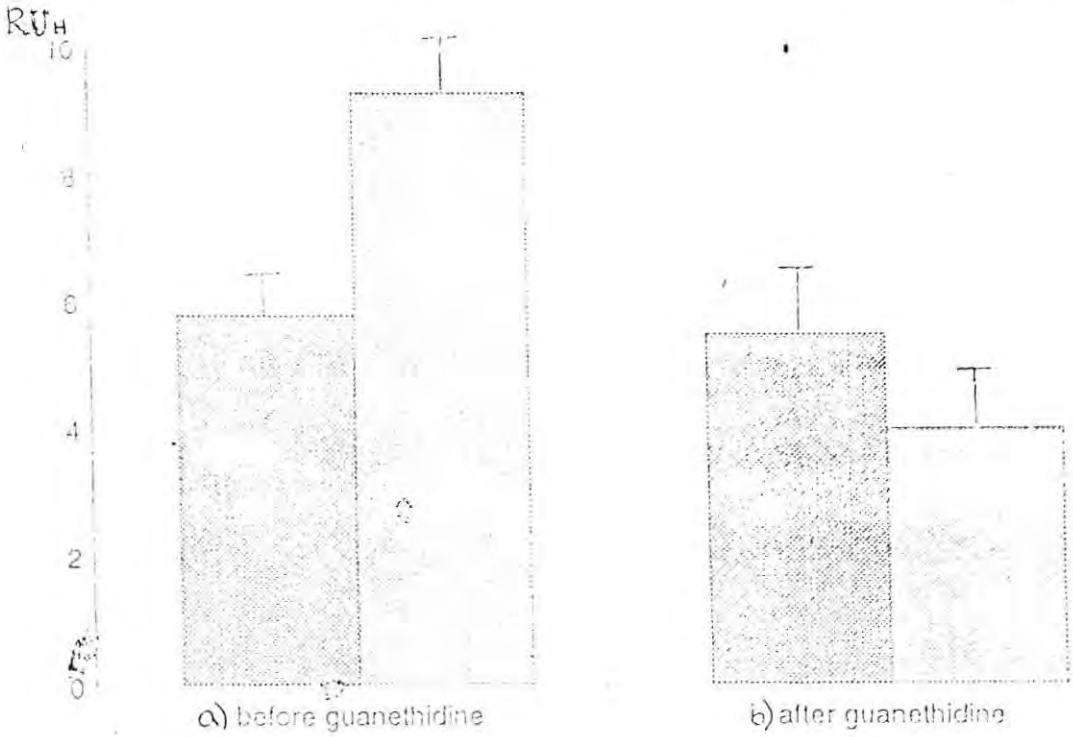


Fig. 4.1.1. Sciatic nerve stimulation-induced hind limb vascular resistance changes before and after guanethidine treatment in anaesthetized and paralyzed rabbits. (n=12, a, $p< 0.001$ b, $p< 0.01$, mean+/- SEM)

Thus, after administration of guanethidine sciatic nerve stimulation induced a reduction of vascular resistance in the hind limb, from 5.5 +/- 1.1 to 4.0 +/- 1.0 RU_H (n=5, p< 0.01, Fig. 4.1.1). This corresponds to a 24.0+/- 2.7 % decrease in vascular resistance during nerve stimulation. The nerve stimulation-induced reduction of hind limb resistance was reproducible (Fig 4.1.3), and was pulse duration, frequency and voltage-dependent (Fig. 4.1.4). The nerve stimulation-induced reductions in hind limb vascular resistance were not affected by atropine (1 mg/kg, n=3) or propranolol (1 mg/kg, n=1)(Figs not shown).

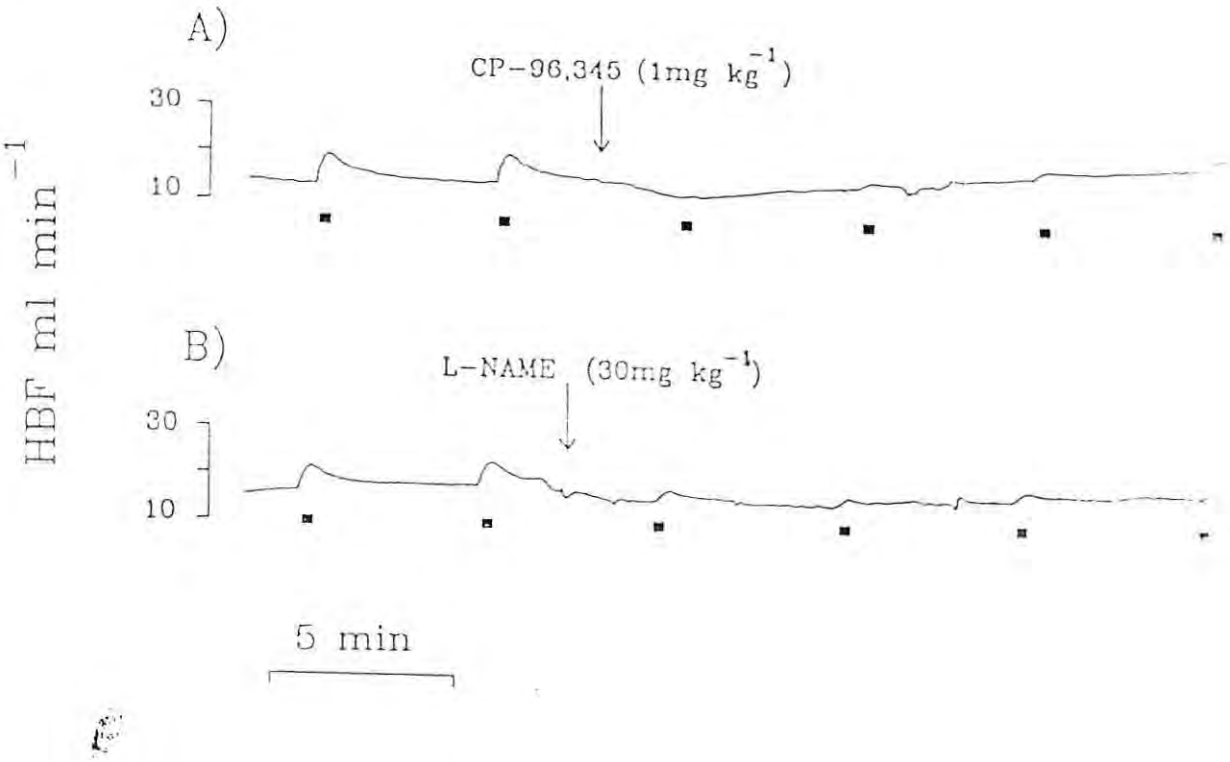


Fig. 4.1.2. The effect of IV infusions of CP-96,345 (1 mg/kg) and L-NAME (30 mg/kg) on nerve-induced changes in hind limb blood flow in anaesthetized, paralyzed and guanethidine treated rabbits. Each denotes nerve stimulation (2Hz, 1ms, 3V and 40 pulses. (n= 5, p< 0.01).

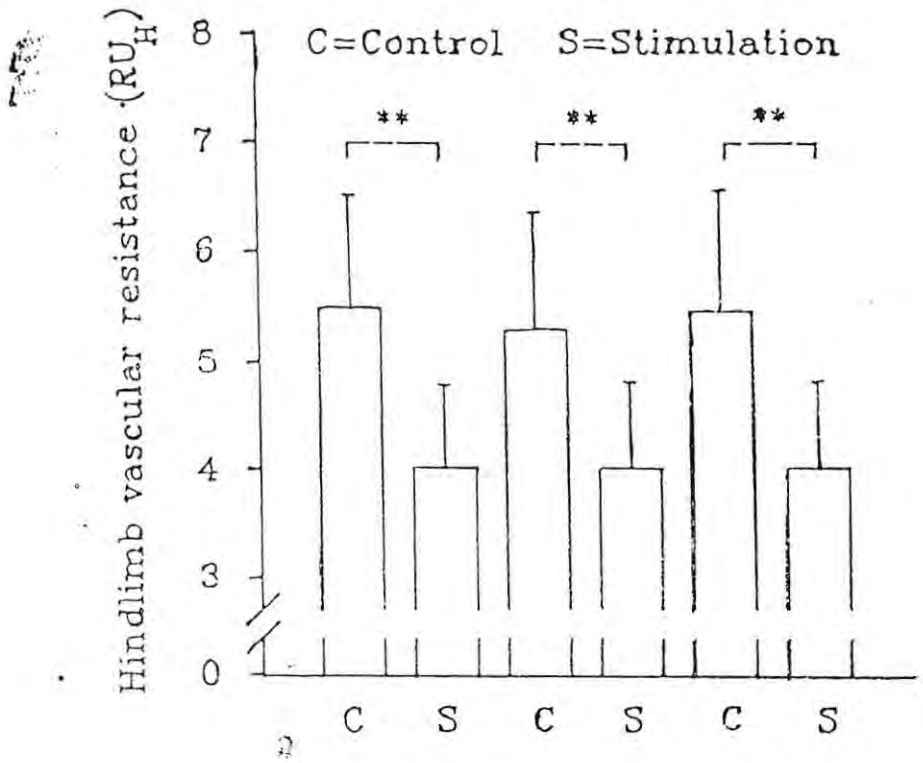


Fig. 4.1.3. Hind limb vascular resistance changes during nerve stimulation and just before the stimulation. **C** denote control values obtained immediately before nerve stimulation. **S** denote the resistance at maximal vasodilation obtained upon nerve stimulation. (n= 5, ** p< 0.01, mean +/- SEM).

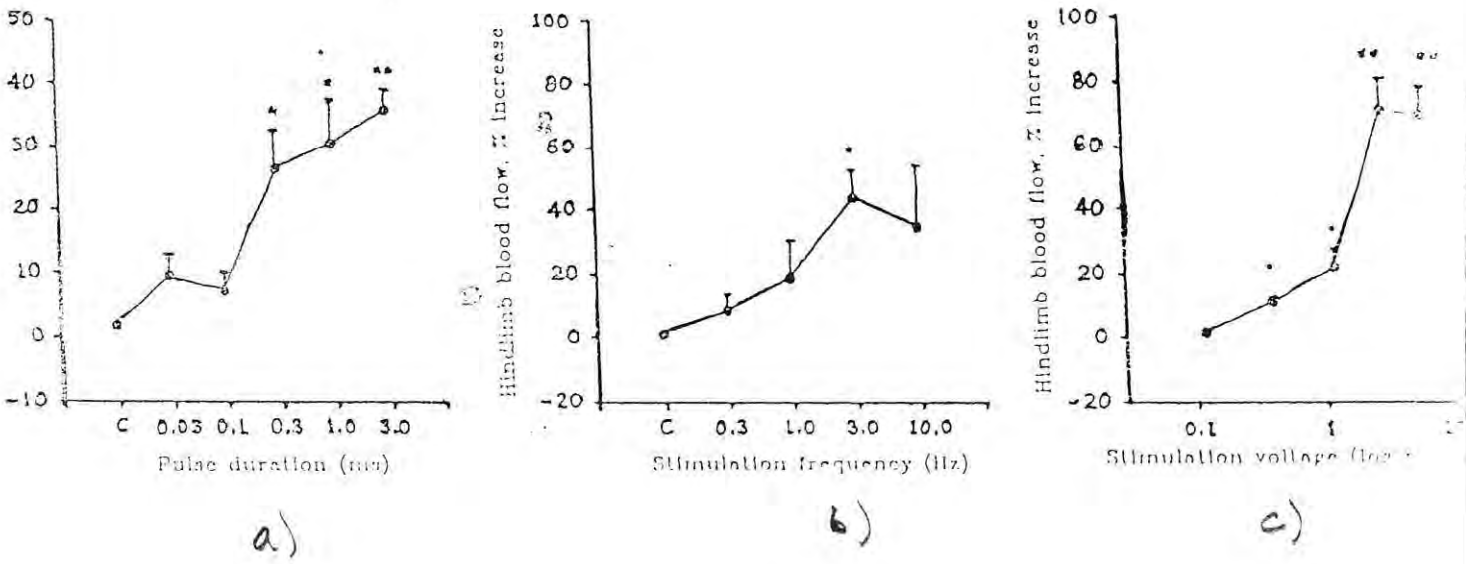


Fig. 4.1.4. Vascular resistance changes during different stimulation parameters a, duration, b, frequency and c, voltage. Vascular responses are expressed as changes in % of unstimulated controls denoted as **C**. (n= 4, * $p< 0.05$, ** $p< 0.01$).

The selective neurokinin-1 receptor antagonist (Snider *et al*, 1991) CP-96,345 (1 mg/kg) antagonised reductions in hind limb vascular resistance induced by intra-arterially administered substance P (10^{-6} M, 100 µl/min) but not those induced by adenosine (2.5×10^{-2}M, 250 µl/min) (Fig. 4.1.5 and Table 4.1.1).

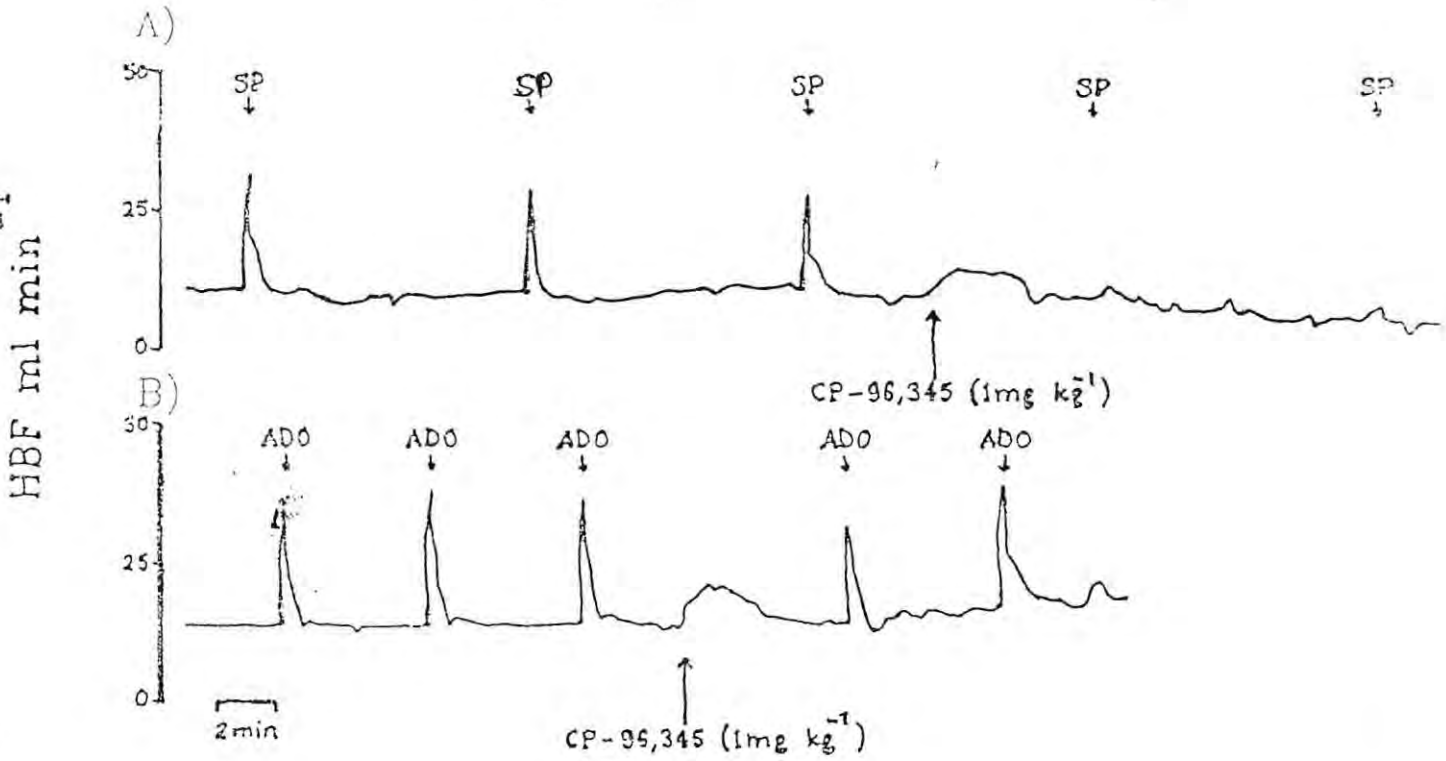


Fig. 4.1.5. Hind limb blood flow changes during close IA infusions of A) SP (10^{-6} M, 0.1 ml/min for 30s) and B) ADO (2.5×10^{-2} M, 0.25 ml/min for 30s), and the effect of IV infusions of CP 96,345 (1mg/kg) on these responses.

In addition, CP-96,345 reduced the nerve-induced vasodilation (Fig.4.1.2). On average the nerve-induced decrease in vascular resistance declined from 22 +/- 3.2 to 4.5 +/- 4.1 % (n=5, p<0.001) by i.v. administration of CP-96,345 (Fig. 4.1.6), indicating the involvement of an endogenous neurokinin-1 receptor agonist in the nerve-induced vasodilation.

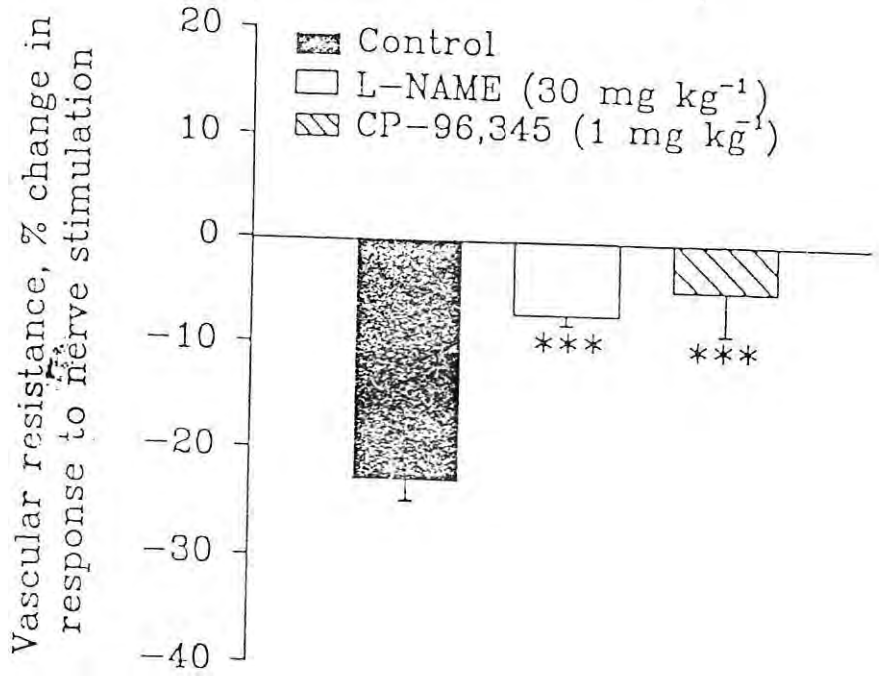


Fig. 4.1.6. The effects of IV infusions of CP-96,345 (1mg/kg) and L-NAME (30mg/kg) on nerve-induced changes in the hind limb vascular resistance [illegible] anesthetized and [illegible] rabbits. [illegible]

CP-96,345 had no significant effect on the basal vascular resistance in the hind limb (Fig. 4.1.7). Upon i.v. infusion, CP-96,345 induced a transient decrease in mean arterial pressure (94.8 +/- 10.4 % of control, p< 0.05, n=5,) (Fig. 4.1.8). However, blood pressure returned close to control values within a few minutes.

L-NAME (30 mg/kg i.v.) increased the basal vascular resistance from 6.0 +/- 0.5 to 9.1 +/- 1.2 RUH (n=5, p<0.05) (Fig. 4.1.7) and it induced an increase in mean arterial pressure by 18 +/- 6% (n=5, P<0.05) (Fig. 4.1.8). In these experiments, in the absence of L-NAME nerve stimulation (parameters as above) induced a decrease in the vascular resistance by 22.6 +/- 2.7 %. The NO synthase inhibitor L-NAME (30 mg/kg) attenuated the nerve-induced increments in femoral flow and the reduction in hind limb vascular resistance was now only 7.0 +/- 1.0% (n=5, p<0.01, Fig. 4.1.2). D-NAME (10-30 mg/kg i.v.) did not attenuate the vascular response to nerve stimulation (n=3) and pretreatment with L-arginine (1 gm/kg i.v.) prevented the effect of L-NAME on the response to nerve stimulation.

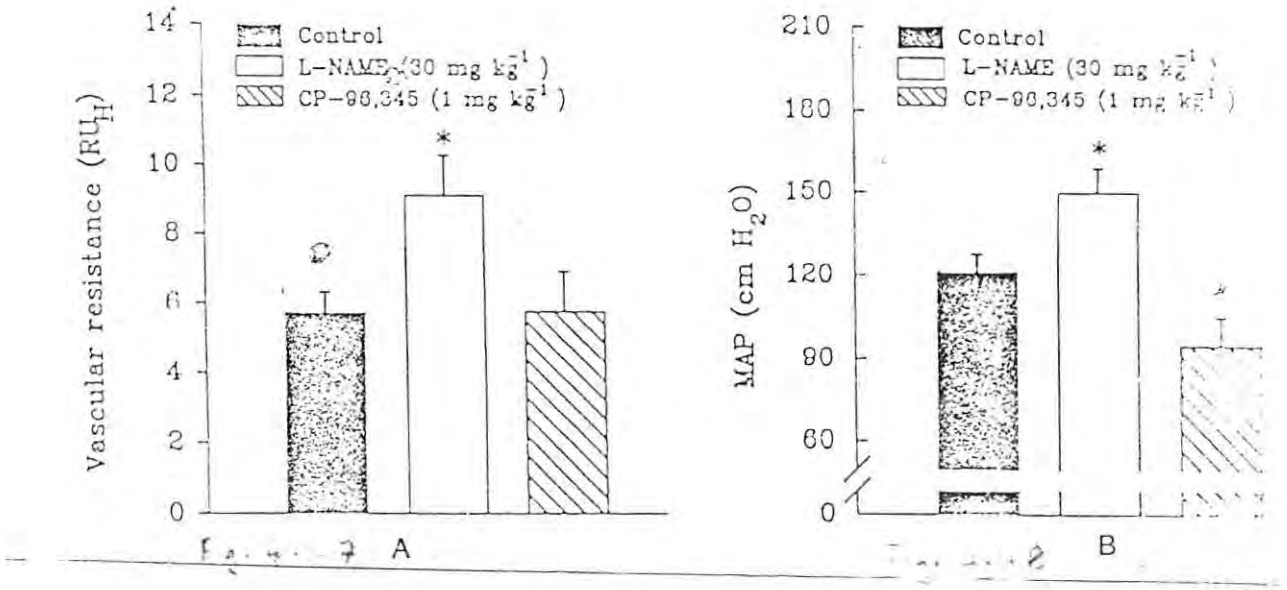


Fig. 4.1.7 and 4.1.8. The effects of CP-96,345 (1mg/kg) and L-NAME (30mg/kg) on hind limb vascular resistance and on the mean arterial pressure in anaesthetized, paralyzed and guanethidine treated rabbits. (n= 5, * p< 0.05, mean +/- SEM).

L-NAME (10 mg/kg) antagonised reductions in hind limb vascular resistance induced by intra-arterially administered substance P (10^{-6} M, 100 µl/min) but not adenosine (2.5 x10^{-2}M, 250 µl/min) (Table 4.1.1).

Tab. 4.1.1. Hind limb vascular resistance changes induced by close IA infusions of SP and ADO before and after treatment with L-NAME (10mg/kg) or CP-96,345 (1mg/kg). Values represent decrease in hind limb vascular resistance (% of resting) by close IA infusions of SP (10^{-6}M, 100 micol/min) or ADO (2.5x 10^{-2}M, 250 micol/min).

	Substance P	Adenosine	n
Pretreatment			
—	58 +/- 6	64 +/- 4	
L-NAME	36 +/- 9*	54 +/- 5	5
—	77 +/- 2	72 +/- 3	
CP-96,345	30 +/- 5***	64 +/- 2	5

Study 2 Intra-cerebroventricular Infusion Studies in the Sheep

The effect of ICV infusion of L-NMMA, an NO-synthase inhibitor, on free water clearance is shown in Fig. 4.2.1. Infusion of L-NMMA at a concentration of 10^{-3}M (20 microL/min) for one hour resulted in increased free water clearance while the infusion of D-NMMA had no significant effect. The effect of L-NMMA started after 40 min of infusion and it reached its peak about ten min after the infusion was stopped. It returned to the pre-infusion level after about fifty minutes. The effect of L-NMMA infused ICV in different concentrations is given in Fig. 4.2.2.

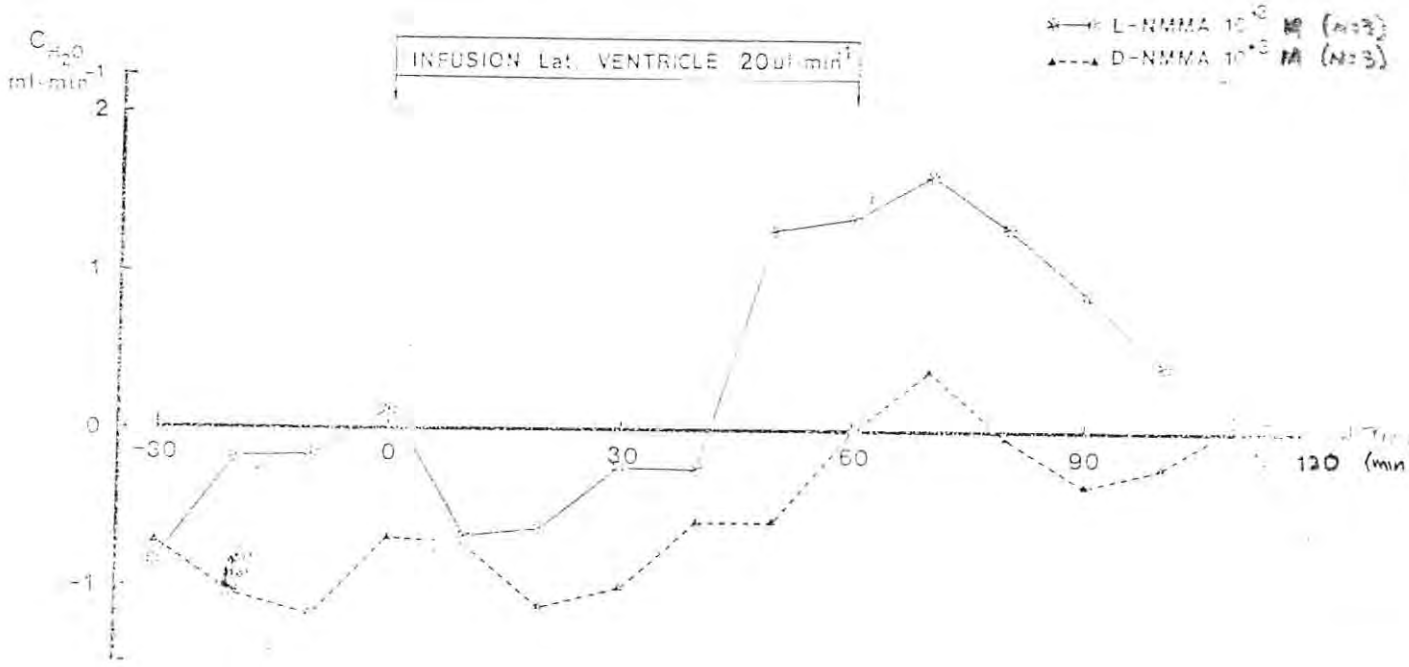


Fig. 4.2.1. Effects of either L-NMMA (10^{-3}M) or D-NMMA (10^{-3}M) in the lateral ventricles on free water clearance (designated C_{H_2O}, ml/min) in the sheep. (n= 3)

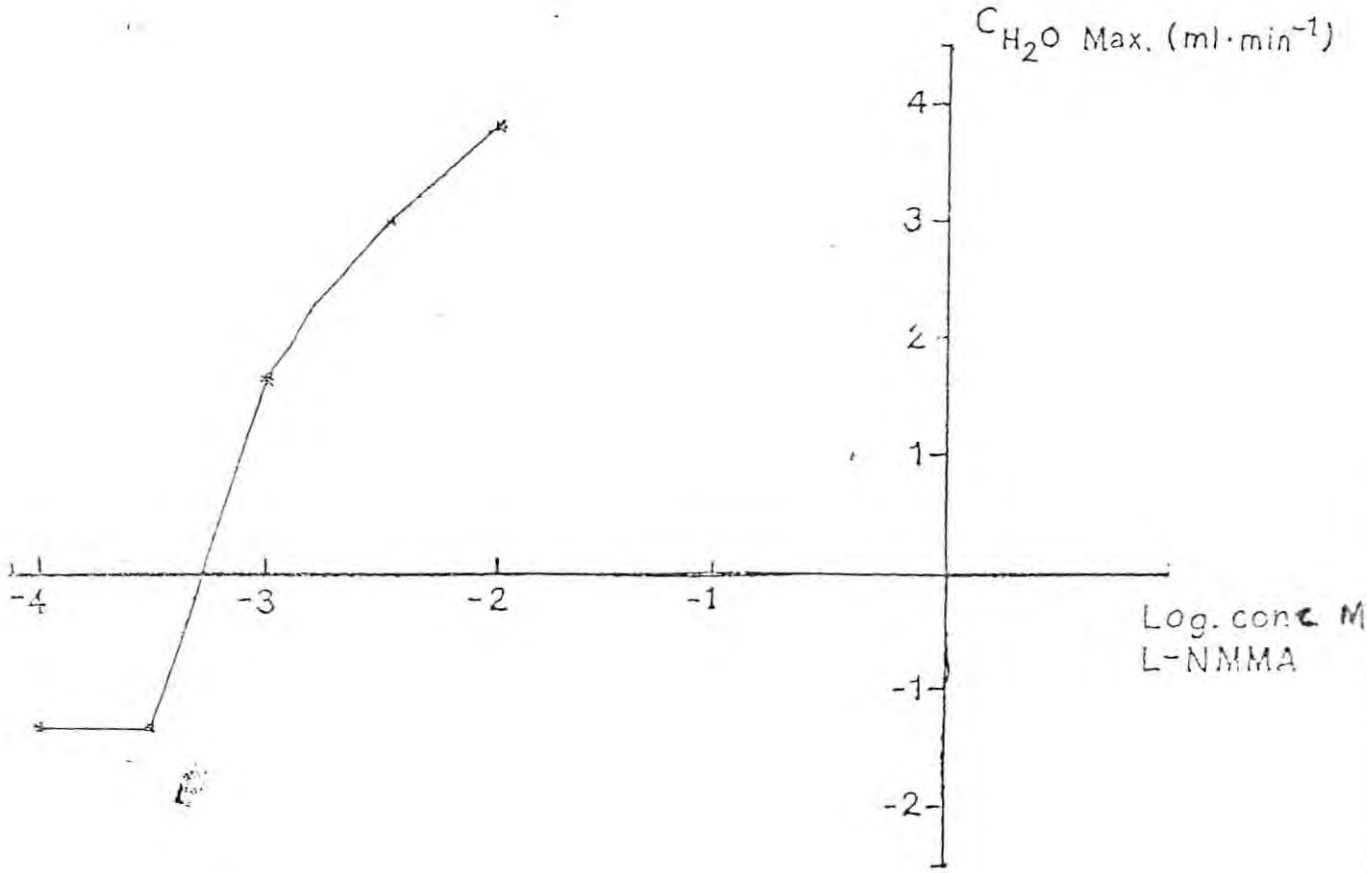


Fig. 4.2.2. The effects of infusing different concentrations of L-NMMA in the lateral ventricles on free water clearance in the sheep (n=5).

The effect of ICV infusion of L-NMMA on plasma AVP levels is shown in Fig. 4.2.3. Plasma AVP level which was 2.7+/- 0.14 pg/ml 30 min before the start of L-NMMA infusion decreased significantly during ICV infusion of L-NMMA to 2.0+/- 0.14 pg/ml ($p < 0.01$) after 60 min. ICV infusion of D-NMMA in the same dose as L-NMMA had no effect on plasma AVP level. Sixty minutes after L-NMMA infusion was stopped the plasma AVP level gradually increased to 2.6 pg/ml.

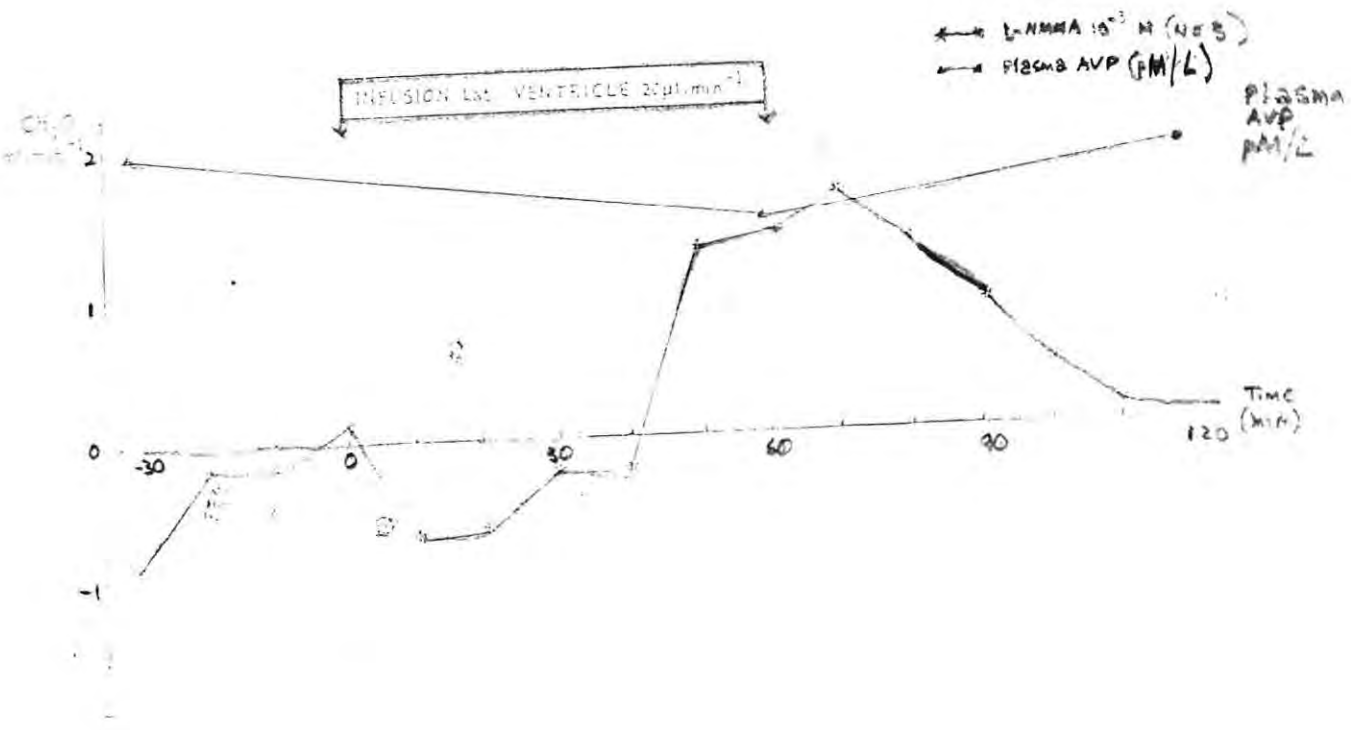


Fig. 4.2.3. Changes in plasma AVP during infusion of L-NMMA (10^{-3}M, 20 micol/min for one hour) in the lateral ventricles of the sheep. (n= 3, $p< 0.01$, mean +/- SEM).

The mean blood pressure and heart rates before, during and after infusion of isotonic L-NMMA is shown in Tab. 4.2.1. Blood pressure decreased significantly from 90 mmHg to 77 mmHg and the heart rate increased from 71/ min, 10 minutes before the start of the infusion, to 88/ min 30 minutes after the infusion was stopped. D-NMMA had no significant effects.

Tab. 4.2.1. Changes in mean arterial blood pressure and heart rate during infusions of L-NMMA (10^{-3}M) and D-NMMA (10^{-3}M) in the lateral ventricles of the sheep (n=3, mean +/- SEM).

PARAMETERS	L-NMMA 10-3M	D-NMMA 10-3M
Mean arterial pressure (mm Hg)		
Control		
-10 min	90	8[illegible]
Infusion period		
30 min	80	8[illegible]
60 min	80	9[illegible]
After infusion		
30 min	83	8[illegible]
60 min	77	7[illegible]
Heart rate (min-1)		
Control		
-10 min	71	7[illegible]
Infusion period		
30 min	72	6[illegible]
60 min	72	6[illegible]
After infusion		
30 min	88	[illegible]
60 min	77	[illegible]

Study 3 Water Deprivation and Rehydration Studies in the Sheep.

The effect of water deprivation and subsequent rehydration on plasma osmolality, sodium, AVP, ANG II levels is given in Table 4.3.1. Plasma osmolality increased significantly ($p< 0.01$) after 96 hours of water deprivation and gradually returned to lower values when water was allowed. This pattern was also observed with plasma sodium changes which increased significantly after 96 hours of water deprivation. Plasma AVP level which was 3.5 +/- 0.9 (control) increased to 9.9+/-5.2 ($p< 0.01$) and decreased when water was allowed. Plasma ANG II increased from 28.6+/-6.6 to 43.1+/-15.7 ($p< 0.01$) and gradually decreased to 36.4+/-4.3 90 min after water was allowed. The relationship of plasma osmolality to plasma AVP is given in Fig 4.3.1. Plasma CGRP and NPY were not significantly altered.

Tab. 4.3.1. The effect of water deprivation and subsequent rehydration on plasma osmolality, sodium, AVP and ANG II levels in sheep. ($n=5$, $p<0.01$) Osmolality in mOsm/kg, Na in mmol/L and neuropeptides in pM/L.

	Osmolality	Na+	AVP	ANG II
control	278.4 ± 5.4	142.7 ± 1.7	3.5 ± 0.9	28.6 ±6.6
48 hrs Dehy.	288.7 ± 4.9	147.3 ± 2.3	3.8 ± 1.5	31.8 ±7.7
96 hrs Dehy.	289 ± 9.2	148.3 ± 2.5	9.9 ± 5.2	43.1±15.7
15 min Rehy.	288.3 ± 9.4	147.8 ± 2.6	5.0 ± 1.9	40.4±8.8
60 min Rehy.	286.9 ± 8.2	147.2 ± 2.6	4.6 ± 1.3	45.8±9.3
90 min Rehy.	283.5 ± 2.1	143.1 ± 1.7	4.1 ± 0.3	36.4±4.3

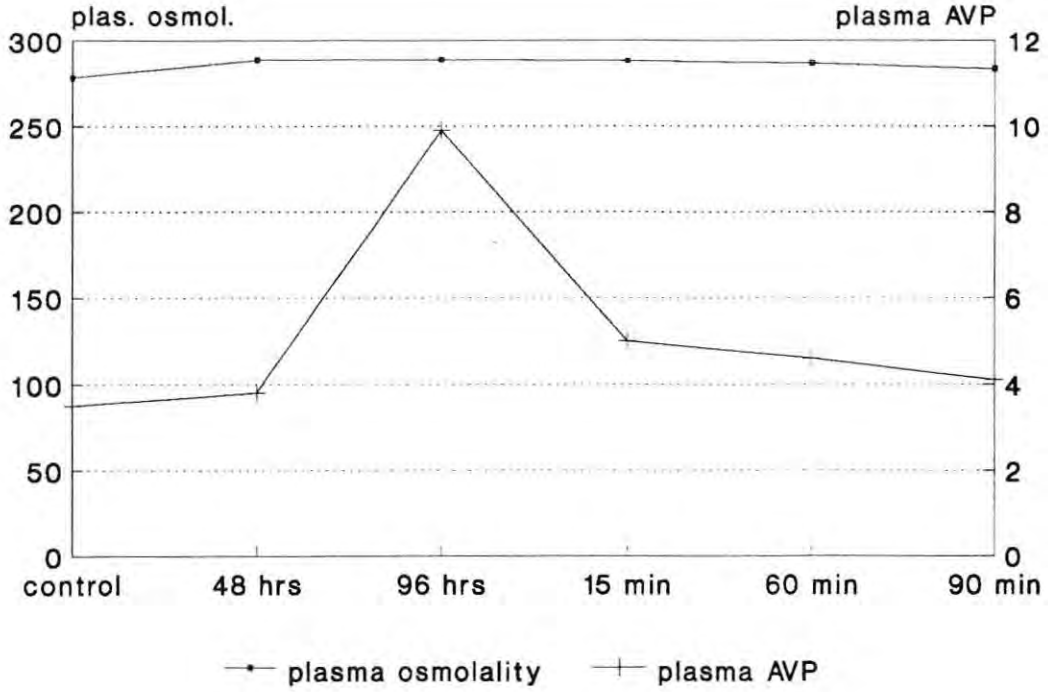


Fig. 4.3.1 The effect of water deprivation and subsequent rehydration on plasma AVP levels in sheep. (n=5,p<0.01) Osmolality in mOsml/kg, AVP in pM/L.

The effect of water deprivation and subsequent rehydration on CSF CGRP, ANG II, and NPY levels is given in Table 4.3.2. The level of CGRP in CSF increased from 260 pM to 315 pM during 96 hrs of water deprivation which gradually returned to lower levels during the subsequent rehydration (n=4, $p< 0.01$). The CSF ANG II which was 79.3+/- 48.3 increased to 620.9+/- 87.2,(n=5, $p<0.001$) after 96 hrs of water deprivation. It gradually decreased to 39.6+/- 28.1 90 min after water was allowed but it was significantly elevated even after 60 min of rehydration ($p< 0.001$). NPY in the CSF increased from 10.2+/- 4.1 to 16.3+/- 4.3 ($p< 0.01$) after 96 hrs of water deprivation and it was significantly elevated even after 15 min of rehydration.

Tab. 4.3.2 The effect of water deprivation and subsequent rehydration on plasma osmolality and CSF levels of ANG II, NPY and CGRP in sheep. (n=5, $p< 0.001$ for ANG II, and $p< 0.01$ for NPY and CGRP, mean +/- SEM). Osmolality in mOsm/L, neuropeptides in pM/L.

	osmolality	**ANG II**	**NPY**	**CGRP**
control	**278.4± 5.4**	**79.3 ± 48.3**	**10.2 ± 4.1**	**260**
48 hrs Dehy.	**288.7± 4.9**	**163.3 ± 42.9**	**16.4 ± 5.8**	**300**
96 hrs Dehy.	**289 ± 9.2**	**620.9 ± 87.2**	**16.3 ± 4.3**	**315**
15 min Rehy.	**288.3± 9.4**	**219.8 ± 36.4**	**19.7 ± 3.3**	**---**
60 min Rehy.	**286.9± 8.2**	**191.3 ± 26.1**	**17.4 ± 4.8**	**---**
90 min Rehy.	**283.5± 2.1**	**39.6 ± 28.1**	**---**	**305**

The relationship of CSF CGRP levels with plasma osmolality is given in Fig. 4.3.2. The increase in plasma osmolality correlated well to the levels of CGRP in CSF. The rise in plasma osmolality was sharper and reached a plateau in shorter time, unlike the gradual rise in cerebrospinal fluid CGRP levels. Both, plasma osmolality as well as cerebrospinal CGRP levels returned to lower levels when animals were hydrated but did not reach to the pre-dehydration levels 90 minutes after the start of rehydration.

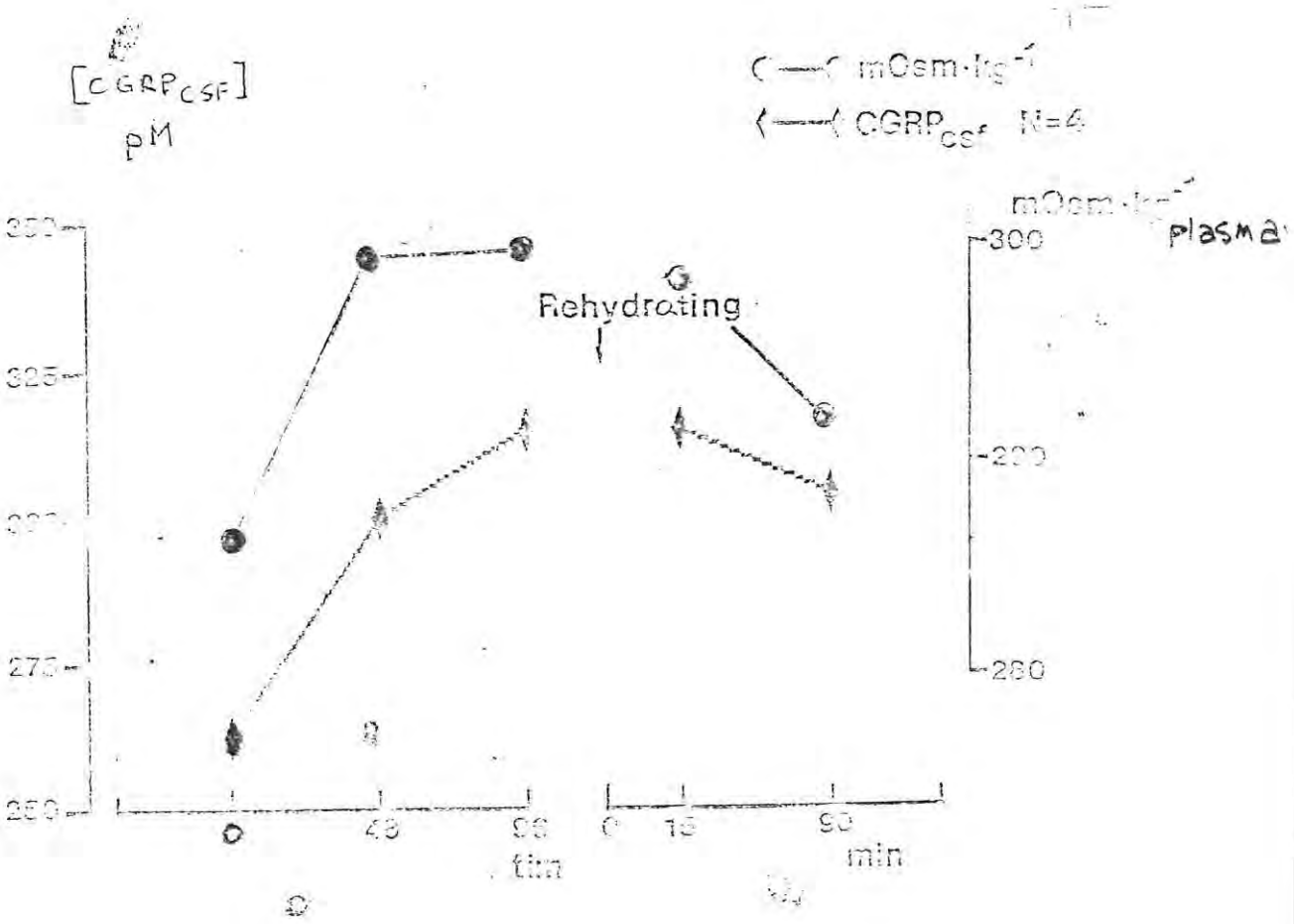
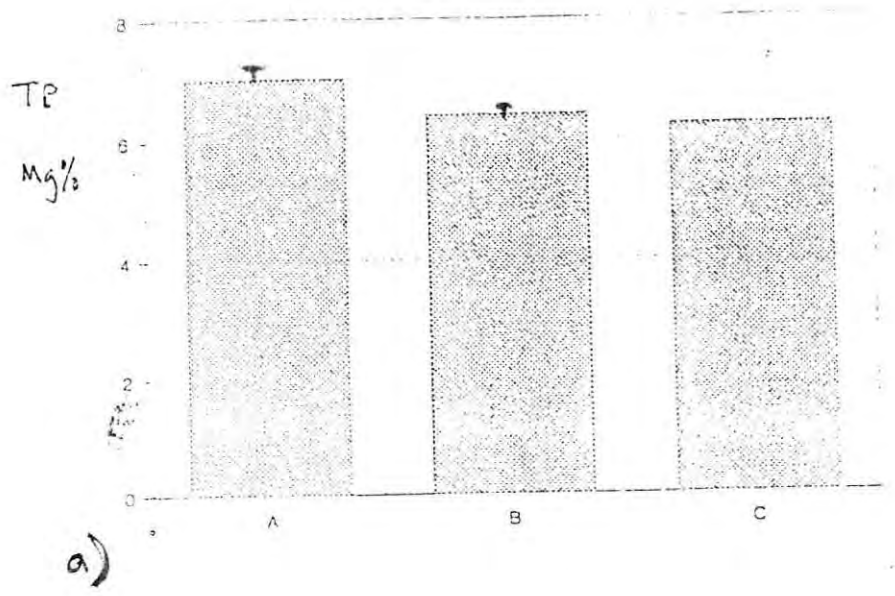


Fig. 4.3.2. The effect of water deprivation and subsequent rehydration on plasma osmolality and CSF CGRP levels. (n=4) CGRP in pM/L and osmolality in mOsm/Kg

Study 4 Studies in Dehydrated Children Secondary to Diarrhoea

Thirteen children ranging from 6 months to 22 months of age were used in this study. Of these five were females and eight were males. From the histories given by the parents, the duration of the diarrhoea ranged from two days to two weeks. Weight of children before the start of ORS treatment ranged from 5 kgs to 12.2 kgs. Weight for age analysis showed that eight out of the thirteen children were below the 5%, four were between 5-10% while only one was at the 50th percentile. All children gained some weight after the ORS treatment. Except one child who needed intravenous therapy and latter maintained on ORS, all others were treated with ORS only, until full hydration was attained. The average duration of rehydration was four hours, ranging from 3-6 hours.

The mean plasma protein level before ORS treatment was 7.02 +/- 0.31 gm% which decreased to 6.4 +/- 0.32 gm% after full hydration (N=9). Both values were higher than controls (Fig. 4.4.1.a). Plasma osmolality decreased from 263.3 +/- 3 mOsm/Kg to 260.9 +/- 2 mOsm/kg after full hydration and these values were lower than the control value which was 273.4 +/- 2 mOsm/kg (Fig. 4.4.1.b). The plasma sodium which was 134.6 mmol/L increased to 148.3 mmol/L after full hydration and plasma potassium increased from 2.78 mmol/L to 3.97 mmol/L after full hydration. Both values, ie, plasma sodium as well as potassium levels before ORS therapy were lower than found in the controls (Fig. 4.4.1.c and d). The analysis of neuropeptides is under process.



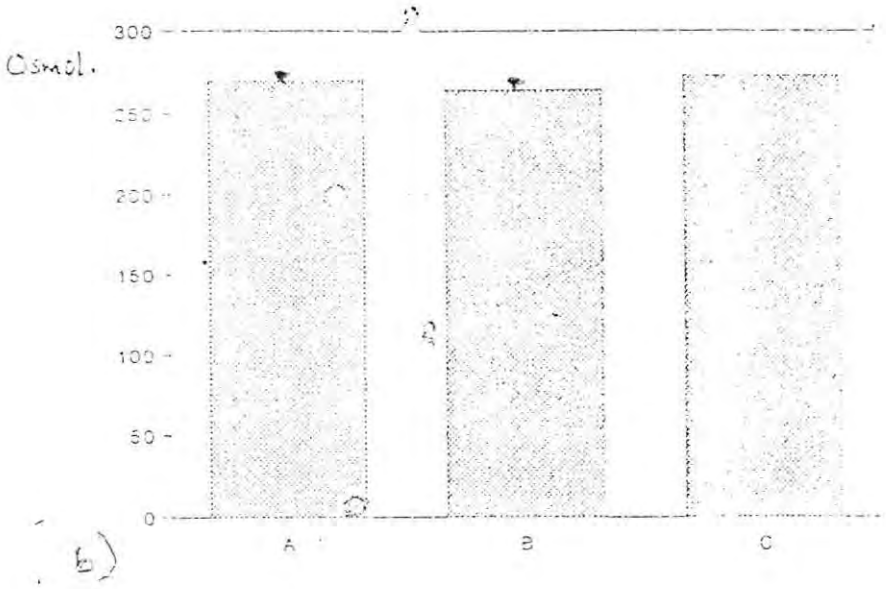


Fig. 4.4.1. Changes in plasma protein a) and osmolality b) before and after ORS therapy in dehydrated children secondary to diarrhoea. (n=9, mean +/- SEM.) Total plasma protein in mg% and osmolality in mOsm/kg. (A = before

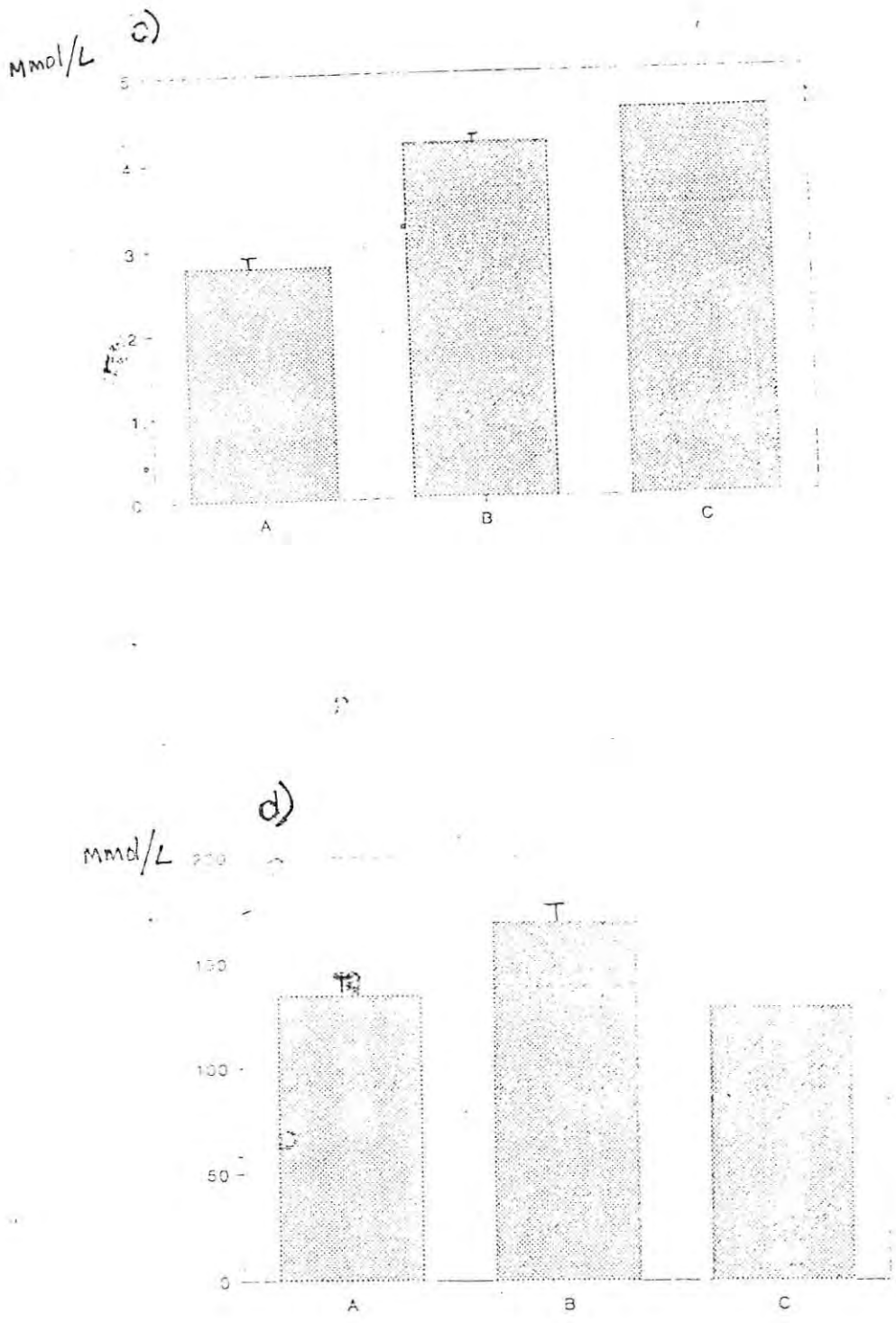


Fig. 4.4.1. Changes in plasma potassium c) and sodium d) before and after ORT treatment in dehydrated children secondary to diarrhoea. (n=9, mean +/- SEM)

CHAPTER V

DISCUSSION

These studies were conducted to analyze the role of various neuropeptides in hydromineral balance and blood flow control in three different species of mammals. The possible involvement of nitric oxide (NO) as a neuro-modulator in the action of these neuropeptides was also studied.

The rabbit model was selected for blood flow studies since stable blood flow recordings could be attained during nerve stimulation for quite a long time with other variables little affected. The sheep, a larger mammal, was used for fluid and electrolyte studies which made it simpler for repeated sample collection, both plasma and CSF, without affecting other variables significantly. Because of the clinical significance of acute diarrhoea in many countries, including that of Ethiopia, a study was conducted in moderately to severely dehydrated children due to diarrhoea. Electrolyte as well as protein level changes in these states were analyzed.

Results obtained in the rabbit experiments indicate the involvement of tachykinin(s) and nitric oxide in the neuro-effector mechanism responsible for vasodilation in response to sciatic nerve stimulation. The nerve induced vasodilation in the hind limb was documented after nor-adrenergic and somato-motor neuro-transmission had been blocked ruling out their possible involvement.

The animals were treated with pancuronium to exclude involvement of muscle contractions and accompanying functional hyperaemia. Electrical stimulation of the sciatic nerve elicited an increase in hind limb vascular resistance (Fig.4.1.1). This was likely to be due to activation of sympathetic vasoconstrictor fibres, since

guanethidine, which inhibits noradrenaline and NPY neuro-transmission, abolished the vasoconstrictor responses to nerve stimulation. In the presence of guanethidine, a vasodilator response was observed (Fig 4.1.2). This vasodilation was unlikely to be secondary to cholinergic mechanisms since atropine failed to affect the response (Data not shown).

The potent, competitive, and highly selective neurokinin-1 (NK-1) receptor antagonist CP-96,345, significantly attenuated the vasodilator response to the nerve stimulation (Fig 4.1.2.A). This suggests that the neurally-mediated vasodilation could either be due to the antidromic stimulation of tachykinin-containing afferent nerve fibres, or stimulation of efferent guanethidine-resistant peptidergic nerves. Tachykinin-containing nerve fibres are shown to project to resistant vessels in rabbit skeletal muscle and are activated by nerve stimulation (Ohlen *et al*, 1987) which is also confirmed in this study. Therefore it is highly probable that tachkinin neuro-transmission was involved in the mediation of the nerve induced vasodilation in this study.

In another set of experiments systemic administration of L-NAME, an NO-synthase inhibitor, diminished the vasodilation secondary to sciatic nerve stimulation to a similar extent as did CP-96,345 (Figs 4.1.2 and 4.1.7). This indicates a role of NO in the nerve stimulation-induced vasodilation. The L-NAME enantiomer, D-NAME did not affect the response to nerve stimulation, and administration of L-arginine, the substrate for NO synthase, prevented the inhibitory effect of L-NAME on nerve stimulation-induced vasodilation. This shows that an enzymatic synthesis of NO from L-arginine was involved in the mediation of the vasodilator response to nerve stimulation.

L-NAME, in addition to causing local increases in vascular resistance, increased the mean arterial blood pressure suggesting the involvement of the L-arginine/ NO pathway in blood flow and blood pressure regulation. This has also been shown in other studies (Rees *et al*, 1990; Persson *et al*, 1990).

If the nerve stimulation-induced reductions in vascular resistance are blocked by CP-96,345 and L-NAME, then it follows that stimulation of the nerve may release a peptidergic neurotransmitter using NO as a mediator. This is further shown by one set of experiments where reductions in hind limb vascular resistance induced by close intra-arterial infusions of SP were attenuated by L-NAME, whereas the reductions in hind limb vascular resistance induced by adenosine were unaffected by L-NAME. These findings suggest that tachykinin receptor-activation and synthesis of NO from L-arginine are involved in the presently observed vascular response to nerve stimulation. This is in agreement with studies which indicate that NO is involved in nerve-induced dilatation of blood vessels, both *in vitro* and *in vivo* (Persson *et al*, 1990; Bult et al, 1990).

The effects of a number of vasodilators, such as SP and Ach, have been shown to be mediated by NO, and NO has been suggested as neurotransmitter in blood vessels (Andersson, 1987). In the present study a tachykinin was likely involved as a primary transmitter since the selective tachkinin receptor antagonist CP-96,345 attenuated the vasodilator response to nerve stimulation. Furthermore, vasodilation induced by the administration of NK-1 agonist, SP, was attenuated by inhibition of NO-synthase. The neurally-induced vasodilation was attenuated by inhibition of NO synthesis. The present data favour the hypothesis that locally synthesized NO mediated the vascular effects of neurally released tachkinin(s) and

indicates that the synthesis of NO is crucial for the nerve-mediated vasodilation in rabbit hind limb. It is likely that NO could be mediating the effect of neurally-released tachykinin receptor agonist(s).

The involvement of neuropeptides in fluid and electrolyte balance, has been studied extensively but the exact mechanism by which these neuropeptides are centrally acting is not known. Peripherally, control of blood flow is achieved by either a direct effect on vascular smooth muscles or via the release of secondary mediators. In this respect nitric oxide has been suggested as a neuro-modulator in connection with many neuropeptides (Johnston *et al*, 1990). The possibility that NO could also be involved in the central regulation of hydromineral balance and neuropeptide release was studied in the sheep experiments. In the present study, ICV infusion of L-NMMA, an NO-synthase inhibitor, resulted in increased free water clearance and a transient decrease in plasma AVP levels but with little effect on blood pressure and heart rate.

This indicates the possibility of NO mediating the release of AVP. D-NMMA, the isomer of L-NMMA, had no significant effects on plasma AVP levels when infused centrally. The fact that plasma AVP level decreased during ICV infusion of L-NMMA suggests that other neuropeptides involved in the central regulation of fluid and electrolyte balance could also be using the L-arginine NO pathway.

Water deprivation is shown to cause a rise in plasma osmolality which is positively correlated with the degree of dehydration, and a number of neuropeptides also increase both in plasma and CSF (Simmon-Opperman *et al*, 1983; Szczepaska- Sadowska *et al*, 1983). In the dehydration study in sheep, water deprivation for 96 hrs resulted in a significant increase in plasma AVP and ANG II

levels which corresponded to the increase in plasma osmolality. All the three variables returned to pre-dehydration levels when water was allowed *ad libitum.* The plasma AVP levels decreased before there was a marked change in plasma osmolality unlike that of ANG II. This is in agreement with earlier observations which suggested that drinking could result in the inhibition of AVP secretion which is of oropharyngeal origin (Davison *et al*, 1988). Plasma levels of CGRP and NPY were not significantly altered during the study. On the other hand, the CSF levels of these neuropeptides as well as that of ANG II increased significantly during water deprivation and corresponded well with plasma osmolality. The CSF level of ANG II and NPY was still elevated after 15-60 minutes of rehydration indicating their slow metabolic rate compared to other neuropeptides like CGRP. The importance of the increase in ANG II in CSF and plasma in maintaining blood pressure and blood flow to vital organs is suggested by some studies (Simmon-Opperman *et al*, 1983).

The importance of the increase in the CSF of the other two neuropeptides is not clearly known. The increase in CGRP levels in the CSF returned to lower levels with rehydration and this corresponded well with the changes in plasma osmolality. Unlike other neuropeptides such as ANG II, which increased in CSF as well as in plasma, CGRP levels taken from plasma didn't change during the dehydration or rehydration period. However, CGRP may participate in the regulation of fluid and electrolyte balance or could just be acting as a neuro-modulator. This neuropeptide is shown to have a strong vasodilatory effect specially in intracranial vessels (Gardiner *et al*, 1991). Unlike its effect when administered peripherally, CGRP causes an increase in blood pressure when administered ICV by increasing plasma NE levels (Breimer *et al*, 1988). So its high level in the CSF in such

conditions may help to increase blood pressure on one hand and adequate blood supply to the brain on the other.

The increase in NPY levels in CSF was rather unexpected since high levels of NPY is known to inhibit sympathetic output centrally and result in hypotension. More studies will be required to show the exact role of NPY in such states.

Unlike the dehydration caused by water deprivation, which usually is attended by plasma hyperosmolality, those caused by diarrhoea in the majority of cases (85%) are isotonic, with a relatively equal loses of fluid and solutes. Only in small number of cases (5-15%) is there a definite hypertonic or hypotonic dehydration due to acute diarrhoea. In children viruses such as rotaviruses and adeno viruses are the commonest causes of diarrhoea which are usually associated with isotonic dehydration.

In the study made in children plasma osmolality, sodium and potassium levels were lower than controls. All variables increased with ORS therapy. Though the data is not large enough to make conclusion on the type of dehydration that occurred in our setting, a hyponatremic dehydration seemed to be the predominant variety. Treatment with dilute home-prepared solutions before coming to the hospital compounded by the pre-existing malnutrition states. The level of dehydration is also shown by the elevated total plasma protein levels which decreased with ORS hydration therapy. Most of the children had clinical signs of some degree of malnutrition and their weight for age in most cases were below the standard, and this *per se* could affect the basal hormonal as well as the electrolyte status of the child. The relatively stable osmolality levels with very low plasma sodium levels increasing during the ORS therapy is interesting since sodium is the major

extracellular cation contributing to ECF osmolality. The increased catabolic rate during such dehydrated states could result in increased organic osmoles from protein catabolism which may have contributed to the maintenance of plasma osmolality within narrow range. The discrepancy between plasma sodium and osmolality after ORS therapy is strange, but the proportion of sodium with water absorbed could affect the picture, but this needs further study to make firm conclusions.

The alteration of neuropeptide level in diarrhoea is being analyzed and this will add invaluable information about the regulatory mechanisms concerning water and electrolytes in such states.

In addition to the studies made above the alteration in neuropeptide levels during diarrhoea could be an interesting area to study and make firm conclusions.

CONCLUSIONS

1. Sciatic nerve stimulation results in increased hind limb blood flow and decreased vascular resistance in a frequency, duration and voltage-dependent manner after neuromuscular and ganglion blockers are applied.

2. Local infusion of substance P or adenosine results in increased blood flow in the rabbit hind limb to a similar extent as sciatic nerve stimulation. The effect of substance P and sciatic nerve stimulation could be blocked by L-NAME without significant effect on adenosine.

3. CP-96,345, a selective neurokinin-1 receptor antagonist abolished the responses to intra-arterial infusion of substance P and to the sciatic nerve stimulation but with little effect on the responses to adenosine administered intra-arterial.

4. Water deprivation for 96 hrs resulted in increased plasma osmolality, arginine vasopressin and, angiotensin II, without a significant increase in plasma CGRP levels. Both cerebrospinal fluid angiotensin II, NPY, CGRP levels and plasma osmolality decreased when water was allowed to be drunk *ad libitum* and the animals rehydrated.

5. Blocking NO, by infusing L-NMMA centrally (ICV), induced a diuresis, with a slight decrease in mean arterial pressure and an increase in heart rate.

6. Plasma levels of arginine vasopressin gradually decreased during central infusion of L-NMMA and slowly increased to pre-infusion levels after the end of the infusion. Nitric oxide seems to affect the release of AVP in the CNS of the sheep. D-NMMA was ineffective in this respect.

7. Unlike the dehydration caused by water deprivation, diarrhoea in the children caused an iso- to hypotonic dehydration, with low Na^+ and K^+ levels. Plasma osmolality, Na^+ and K^+ levels increased with ORS therapy.

8. Neuropeptides act centrally as well as peripherally to regulate hydromineral balance and blood flow. Nitric Oxide, released from L-arginine, seems to have an important neuro-modulatory role in this aspect.

REFERENCES

Ahren, B. (1989). Effects of calcitonin, katakalcin and CGRP on basal and TSH stimulated thyroid hormone secretion in the mouse. Acta Physiol. Scand.**135**: 133-137.

Allen, D.E. and Gellai, M. (1987). Cardio-inhibitory effect of atrial peptide in conscious rats. Am.J.Physiol.**252**: R610-R616.

Almeida, F.A., Suzuki, M., Scarborough, R.M., Lewiicki, J.A. and Maack, T. (1989). Clearance function of type C receptors of atrial natriuretic peptide in rats. Am.J.Physiol.**256**: 469-475.

Andersson, B. (1978). Maintenance of water balance depends on a sensitive regulation of the release of ADH on one hand and on an efficient drinking on the other. Physiol.Rev.**60**: 961-1048.

Andersson, S.E. (1989). Effects of intravenous calcitonin-gene-related peptide (CGRP) on local blood flow in the cat. Acta Physiol.Scand.**137**: 259-70.

Andersson, S.E. (1987). Responses to antidromic nerve stimulation, substance P, neurokinin A, calcitonin-gene-related peptide and capsaicin in the rat eye. Acta Physiol.Scand.**131**: 371-376.

Anthone, G.J., Orandle, M.S., Marcophilips, Z.V., Wang, B.H., and Yeo, C.J. (1991). Neuropeptide Y is a pro-absorptive agent in the small intestine. J.Sur.Res.**50**: 589-94.

Antonia, L. and Burnstock, G. (1991). Endothelium-dependent and endothelium-independent vasodilation of the rabbit hepatic artery. Br.J. Pharmacol.**103**: 1206-12.

Applegren, B., Arver, S., Sagulin, G.B. (1986). Effect of intracerebroventricular calcitonin on renal hydromineral excretion in sheep. Am.J.Physiol.**250**: 980-983.

Applegren, B., Eriksson, S., and Kopp, S. (1991). An experimental model of temporo-mandibular joint arthritis in sheep. (paper submitted).

Arjamaa, O., Karlqvist, K., Karervo, A., Vainionpaa, V., Vuolteenaho, O. and Leppaluoto, J. (1992). Plasma ANP during hypertonic NaCl infusion in man. Acta Physiol. Scand.**144**: 113-119.

Bargmann, W. and Scharrer, E. (1951). The site of origin of the posterior pitutary. Am. Sci.**39**: 225-259.

Beglinger, C., Koehler, E., Born, W., Fischer, J.A., Keller, U., Hanssen, L.E. and GYR, K. (1988). Effect of calcitonin and calcitonin-gene-related peptide on pancreatic functions in man. J.British Soc. of GE. **29**: 243-248.

Ben-Ari, E.T. and Garrison, J.C. (1988). Regulation of angiotensinogen mRNA accumulation in rat hepatocytes. Am.J.Physiol. **255**: 70-79.

Bestle, M.H. and Bie, P. (1991). Renal effects of urodilantin and ANP in volume expanded conscious dogs. Abstract.

Bie, P. (1980). Osmoreceptors, vasopressin and control of renal water excretion. Physiol.Rev. **60**: 961-1048.

Blair-West, J.R., Denton, D.A., McKinley, M.J., and Weisinger, R.S. (1988). Angiotensin-related sodium appetite and thirst in cattle. Am.J.Physiol. **24**: 205-211.

Brain, S.D. and Williams, T.J. (1988). Substance P regulates the vasodilator activity of calcitonin-gene-related peptide (CGRP). Nature.**335**: 73-75.

Brain, S.D., and Williams, T.J. (1985). Inflammatory oedema induced by synergism between calcitonin-gene-related peptide and mediators of increased vascular permeability. Br.J.Pharmacol. **86**: 855-860.

Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R. and MacIntyre, I. (1985). Calcitonin-gene-related peptide (CGRP) is a potent vasodilator. Nature.**313**: 54-56.

Breimer, L.H., MacIntyre, I. and Zaidi, M. (1988). Peptides from the calcitonin genes: molecular genetics, structure and function. Biochem. J. **255**: 377-390.

Brenner, M.B., Ballermann, B.J., Gunning, M.E., and Zeidel, M.L. (1990). Diverse biological actions of atrial natriuretic peptide. Physiol. Rev. **70**: 665-669.

Breuhaus, B.A., Saneii, H.H., Brandt, M.A. and Chimoskey, J.E. (1987). Atriopeptin II lowers cardiac output in conscious sheep. Am.J.Physiol. **249**: 776-780.

Brown, G.P. and Venuto, R.C. (1988). Angiotensin II receptors in rabbit renal pre-glomerular vessels. Am. J. Physiol.**255**(1): 16-22.

Brown, G.P. and Venuto, R.C. (1991). Renal and blood flow responses to ANG II infusions in conscious pregnant rabbits. Am. J.Physiol.**261**: 51-59.

Buckley, T.L., Brain, S.D., Ramport, M. and Williams, T.J. (1991). Time-dependent synergistic interactions between the vasodilator neuropeptide calcitonin gene related peptide (CGRP) and mediators of inflammation. Br.J.Pharmacol.**103**: 1515-1519.

Bult, H., Boeckxtaens, G.E., Pelkmans, P.A., Jordaens, F.H., Van Maercke, Y.M., Herman, A.G. (1990). Nitric Oxide as an inhibitor non-adrenergic non-cholinergic neurotransmitter. Nature. **345**: 346-7.

Burnett, J.C., Jr., Granger, J.P., Opgenorth, T.J. (1984). Effects of atrial natriuretic factor on renal function and renin release. Am. J. Physiol. **247**: 863-866.

Burnett, J.C. Jr., Kao, P.C., Hu, D.C., Hesser, D.W., Heiublein, D., Reeder, G.S., and Opgenorth, T.J. (1986). Atrial natriuretic peptide in congestive heart failure in the human. Science. **231**: 1145-1147.

Carlisle, L., Aberden, J., Forge, A. and Burnstock, G. (1990). Neural basis for regulation of cochlear blood flow: peptidergic and adrenergic innervation of spiral modiolar artery of the guinea pig. Hear Res. **43**: 107-13.

Chang, M.M., Leeman, S.E. and Niali, H.D. (1971). Amino acid sequence of Substance P. Nature. **232**: 86-87.

Chen, X., Knuepfer, M.M. and Westfall, T.S. (1990). Hemodynamic and sympathetic effects of spinal administration of Neuropeptide Y in rats. Am.J.Physiol. **259**: 1, 1674-1680.

Clark, I.A., Rockett, K.A., Codwen, W.B. (1992). Possible central role of nitric oxide in conditions clinically similar to cerebral malaria. Lancet. **340**: 894-96.

Clausen, T. and Andersson, S.V. (1991). Calcitonin and Calcitonin-gene-related peptide, (CGRP), stimulate active Na-K transport in rat skeletal muscle. Abstract.

Cody, R.J., Atlas, S.A., Laragh, J.H., Kubo, S.H. (1986). Atrial natriuretic peptide levels in normal and heart failure patients: plasma level,renal,hormonal and hemodynamic responses to peptide infusion. J.Clin.Invest. **78**: 1362-1374.

Conley, S.B. (1990). Hypernatremia. Paediatr-Clin-North-Am. **37**: 365-72

Cowley, A.W. Jr., Switzer, S.J. and Guinn, M.M. (1980). Evidence in quantification of the vasopressin control system in the dog. Cir. Res. **46**: 58-67.

Crossman, D.C., McEwan, J., MacDermot J., MacIntyre, I., and Dollery, C.T. (1987). Human CGRP activates adenylate cyclase and releases prostacyclin from human umbilical vein endothelial cells. Br.J. Pharmacol.**92**: 695-701.

Dahlborn, K. and Holtenins, K. (1991). Infusion of ANP decrease food intake in the goat. abstract.

Davison, J.M., Shiells, E.A., Philips, P.R. and Lindheimer, D.M. (1988). Suppression of AVP release by drinking despite hypertonicity during and after gestation. Am.J. Physiol. **254**: 588-592.

De Bold, A.J., Borenstein, H.B., Veress, A.T., Sonnenberg, H. (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Life Science.**28**: 89-94.

Demman, J.G., Palkmans, P.A., Boeckxtaens, G.E., Bult, H., Oosterbosch, L., Herman, A.G. and Van Maercke, Y.M. (1991). The role of nitric oxide in inhibitory non-adrenergic non-cholinergic neurotransmission in the canine oesophageal sphincter. Br.J.Pharmacol.**103**: 1092-6.

Edvinsson, L., Ekbald, E., Hakanson, R. and Wahlestedt C. (1984). Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br.J.Pharmacol.**83**: 519-525.

Edwards, B.S., Schwab, T.R., Zimmerman, R.S., Heinublein, D.M., Jiang, N.S. and Berneth, J.C. Jr. (1986). Cardiovascular, renal and endocrine responses to ANP in ANG II mediated hypertension. Cir.Res. **59**: 663-667.

Ellenbogen, K.A., Rogers, R., Walsh, M., Mohanty, P.K. (1988). Increased circulating atrial factor (ANP) release during induced-ventricular tachycardia. Am. Heart. J. **116**: 1233-38.

Epsten, M., Loutzenheiser, R., Friedland, E., Aceto, R.M., Camargo, M.J. and Atlas, S.A. (1987). Realationship of increased atrial natriuretic factor and renal sodium handling during immerssion-induced central hypervolemia in normal humans. J.Clin. Investigation.**79**: 738-745.

Eriksson S., Simon-Opperman, Ch., Simon, E. and Gray, D.A. (1988). Occlusion of rostraventral third ventricle abolishes drinking but not AVP release in response to central osmotic stimulation. Brain research. **448**: 121-127.

Fahrenkrug J. et al (1989). Regulatory peptides in the mammalian urogenital system. Expermentia-Suppl. **56**: 362-81.

Fayad, I.M., Hirsccorn., Abu-Zikry, M., Kamel, M. (1992). Hypernatremia surveilance during a national diseases control project in Egypt. Lancet. **339**: (8790) 389-93.

Franco-Cereceda, A. and Lundberg, J.M. (1987). Potent effects of NPY and CGRP on human vascular tone *in vitro*. Acta Physiol. Scand.**131**: 159-160.

Franko-Cereceda, A., Lundberg, J.M., Saria, A., Schreibmayer, W. and Trithart, H.A. (1988). CGRP: release by capsaicin and prolongation of the action potential in the guinea-pig heart. Acta Physiol. Scand.**132**: 181-190.

Franko-Cereceda, A., Rudehill, A. and Lundberg, J.M. (1987). Calcitonin-gene-related peptide not substance P mimics capsaicin-induced coronary vasodilatation, in the pig. Eur.J.Pharmacol.**142**: 235-43.

Franco-Cereceda, A., Saria, A. and Lundberg, J.M. (1987). Ischemia and related changes in contractility induced release of CGRP but not NPY from isolated perfused guinea-pig heart. Acta Physiol. Scand.**131**: 319-320.

Fuller, R.W., Conradson, T.B., Dixon, C.M.S., Crossman, D.C. and Barnes, P.J. (1987). Sensory neuropeptides, effects in human skin. Br.J.Pharmacol.**92**: 781-788.

Garcia, R., Debniski, W., Gutkowski, J., Kuchel, O., Thibault, G., Genest, J. and Cantin, M. (1985). Gluco- and mineralocorticoids may regulate the natriuretic effect and synthesis and release of atrial natriuretic factor by the rat atria *in vitro*. Bioch.Biophy.Res.Commun.**131**: 806-814.

Gardiner, S.M., Campton, A.M. and Bennett, T. (1989). Regional hemodynamic effects of calcitonin-gene-related peptide. Am.J.Physiol. **256**: R332-8.

Gardiner, S.M., Campton, A.M., Kemp, P.A., Bennett, T., Foulkes, R. and Hughes,B (1991). Regional hemodynamic effects of prolonged infusions of human alfa-CGRP in conscious, Long Evans rats. Br.J. Pharmacol.**103**: 1509-1514.

Gray, D.A. and Simon, E. (1985). Control of plasma angiotensin II in a bird with salt glands (*Anas platyrhynchos*). Gen Comp Endocrinol.**60**: 1-13.

Greenberg, B., Rhoden, K. and Barnes, P.J. (1987). Calcitonin- gene -related peptide is a potent non-endothelium dependent inhibitor of coronary vasomotor tone. Br. J.Pharmacol. **92**: 789-794.

Greenley, G.H. Jr., Luis, F., Gomez, G., Ishizuka, J., Holland, B. and Thompson, J.C. (1988). Peptide YY antagonizes beta-adrenergic-stimulated release of insulin in dogs. Am.J.Physiol. **17**(4): E513-E517.

Gregory, C.L., Quillen, E.W. Jr., Keil, L.C., Chang, D. and Reid, I.A. (1988). Effect of vasopressin blockade on blood pressure during water deprivation in intact and baroreceptor-denervated conscious dogs. Am.J.Physiol.**17**: 490-495.

Gustafsson, L.E., Wiklund, C.U., Wiklund, N.P., Persson, M.G., Moncada, S. (1990). Modulation of autonomic neuroeffector transmission by nitric oxide in guinea-pig ileum. Biochem Biophys Res Commun. **173**: 106-110.

Hall, J.E., Montani, J.P., Woods, L.L. and Mizelle H.L. (1988). Role of vasopressin and pressure diuresis in the regulation of AP and body fluid volume. In vasopressin.

Harfstrand, A., Lundberg, J.M., and Fuxe, K. (1987). NPY receptors in pig spleen. Acta Physiol.Scand.**131**: 321-322.

Henrich, W.L., McAllister, E.A., Smith, P.B., Campbell, W.B. (1988). Guanosine 3', 5'-cyclic mono phosphate (cGMP). as a mediator of inhibition of renin release. Am.J.Physiol.**255**: F474-F478.

Henrich, W.L., McAllister, E.A., Smith, P.B., Lipton, J. and Campbell, W.B. (1987). Direct inhibitory effect of atriopeptin II on renin release in primate kidney. Life science.**41**: 259-264.

Henrich, W.L., Needleman, P. and Campbell, W.B. (1986). Effect of atriopeptin II on renin release *in vitro*. Life Science. **39**: 993-1001.

Hernandez, M.F. and Morales-Barradas, J.A. (1990). Sodium concentrations for oral rehydration in children with diarrhoea. Bol-Med-Hosp-Infant-Mex. **47**: 285-91.

Hildebraudt, D.A. and Banks, R.O. (1988). Effect of ANP on renal function in rats with nephrotic syndrome. Am.J.Physiol. **254**: F210-F216.

Hock, C.E., Sue, J.Y. and Lefer, A.M. (1984). Role of AVP in the maintenance of circulatory haemostasis during haemorrhagic shock. Am.J.Physiol. **246**: H174-179.

Hodsman, G.P., Tsunoda, K., Ogawa, K., Johnston, C.I. (1985). Effects of posture on circulating atrial natriuretic peptide. Lancet.**2**: 1427-1429.

Holzer-Perche, U., Petritsch, W., Hinterleinter, T., Eherer, A., Sperk, G., Krejs, G.J. (1991). Effect of Neuropeptide Y on jejunal water and ion transport in humans. Gastroenterology.**101**: 325-30.

Hua, X.Y. (1986). Tachykinins and CGRP in relation to peripheral functions of capsaicin- sensitive sensory neurons. Acta Physiol.Scand. (suppl.551, 1-45).

Ignarro, F., Jolou-Schaeffer, G., Gray, G.A., Parrat, J.R., Stoclet, J.C. (1991). Evidence that an L-arginine/ NO dependent elevation of tissue cyclic GMP content is involved in depression of vascular reactivity by endotoxins. Br.J.Pharmacol.**103**: 1047-1052.

Ignarro, L.J. (1990). Nitric Oxide, A novel signal transduction mechanism for transcellular communication. Hypertension. **16**: 447-483.

Irwin, M., Brown, M., Paterson, T., Hanger, R., Mescovich, A. and Grant, I. (1991). Neuropeptide Y and natural killer cell activity: findings in depression and Alzheimer care-giver stress. The Faseb Journal.**5**: 3100-3107.

Iwao, H., Fukni, K., Kim, S., Nakayama, K., Ohkubo, H., Nakamishi, S. and Abe, A. (1988). Sodium balance effects on renin, angiotensinogen and atrial natriuretic peptide levels. Am.J.Physiol.**18**. 129-136.

Janiak, P. and Brody, M.J. (1988). Central interactions between aldosterone and vasopressin on cardiovascular system. Am.J. Physiol.**255**(1): R166-R173.

Jernbeck, J., Edner, M., Dalsgard, C.J. and Pernow, B. (1990). The effect of calcitonin-gene-related peptide (CGRP) on human forearm blood flow. Clinical Physiol.**10**(4): 335-43.

Johnston, F.G., Bell, B.A., Robertson, I.J.A., Miller, J.D., Haliburn, C., O'Shaughnessy, D., Riddel, A.J. and O'Laoire, S.A. (1990). Effect of CGRP on postoperative neurological deficits after subarachnoid haemorrhage. Lancet. **335**: 869-872.

Karlsson, J.A., Zackrisson, C. and Lundberg, J.M. (1991). Hyper-responsiveness to tussive stimuli in cigarette smoke-exposed guinea pigs: a role for capsaicin-sensitive CGRP containing nerves. Acta Physiol. Scand. **141**: 445-454.

Kawasaki, H. (1988). CGRP acts as a novel vasodilator neurotransmitter in mesenteric resistance vessels of the rat. Nature.**335**: 8 Sep.

Kenneth, A., Ellenbogen Mohanty, P.K., Sowers, J.R., Walsh, M. and Thames, M.D. (1988). ANP release is enhanced by incremental atrial pacing. Am. heart J. **116**: 489-496.

Knight, K.R., et al (1988). Effect of the vasodilator peptides CGRP and atriopeptin on rabbit microvascular blood flow (preliminary communication). Br.J.Plastic Surg. **41**: 147-9.

Lappe, R.W., Todt, J.A. and Wendt, R.L. (1987). Effect of Atrial natriuretic peptide on the vasoconstrictor actions of the renin-angiotensin system in conscious rats. Cir.res. **61**: 134-140.

Larsen, P.J., Jessop, D.S., Chodrey, H.S., Mikkelsen, J.D., Lightman, S.L. (1992). Osmotic regulation of substance P and neurokinin A peptide content and substance P binding sites in distinct hypothalamic nuclei of the rat. Peptides. **13**: 705-12.

Lattion, A.L., Jean-Baptiste, M., Arenald, E., Corvol, P. and Sonbrier, F. (1986). Myocardial recruitment during ANP mRNA increases with volume overload in the rat. Am.J.Physiol. **251**: H890-896.

Ledsome, J.R., Wilson, N., Courneya, C.A. and Rankin, A.J. (1985). Release of atrial natruretic peptide by atrial distension. Can.J.Physiol.Pharmacol. **63**: 739-742.

Lee, S.L., Hoglundhal, A.U. and Sjoquist, M. (1991). Influence of vasopressin V_2-receptor antagonist on CNS-induced natriuresis. Abstract.

Lenz, H.J., Mortrund, M.T., Rivier, J.E. and Brown, M.R. (1985). Calcitonin-gene-related peptide inhibits basal, pentagastrin, histamine, and bethanecol stimulated gastric acid secretion. Gastroenterology.**26**: 550-555.

Leung, F.W., et al (1987). CGRP inhibits acid secretion without modifying blood flow. Am. J. Physiol.**252**: 215-8.

Lind, W.R., Swanson, L.W. and Ganten, D. (1985). Organization of Angiotensin II immunoreactive cells and fibres in the rat central nervous system. Neuroendocrinology.**40**: 2-24.

Lishajko, F. (1983). Separation and purification of blood plasma arginine vasopressin on Sephadex G-50M for subsequent radio-immunoassay. Acta Physiol. Scand.**118**: 361-367.

Lowenstein, C.J. and Snyder, S.H. (1992). Nitric Oxide a novel biologic messenger. Cell. **70**: 705-707.

Lulseged, S. (1992). Predictors of modrate to severe dehydration in acut diarrhoeal disease: a case control study. Ethiop. Med-J.**30**: 61-78.

Lundberg, J.M., Franco-Cereceda, A., Hua, X., Hokfelt, T. and Fisher, J.A. (1985). Co-existence of substance P with calcitonin-gene-related peptide-like immuno-reactivities in sensory nerves in relation to cardio-vascular and bronchoconstrictor effects of capsaicin. European J. Pharmacol.**108**: 315-319.

Lundberg, J.M., Fried, G., Pernow, J. and Theodorsson-Norheim, E. (1986). Co-release of Neuropeptide Y and catecholamines upon adrenal activation in the cat. Acta Physiol. Scand.**126**: 231-238.

Lundberg, J.M. and Tatemoto, K. (1982). Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. Acta physiol. Scand.**116**: 393-402.

Luu, T.N., Chester, A.H., O'Neil, G.S., Tadjkarimi, S., Yacoub, M.H. (1992). Effects of vasoactive neuropeptides on human saphenous vein. Br.heart.J.**67**: 474-7.

MacDoland, N.J., et al (1989). A comparison of the effects of human CGRP and glyceryl trinitrate on regional blood velocity in man. Br.J.Clin. Pharmacol.**28**: 257-61.

Maisel, A.S., Scott, N.A., Motulsky, H.J., Michel, M.C., Bonblik, J.K., Rivier, J.E., Ziegler, M., Allen, R.S. Brown, M.R. (1989). Elevation of NPY levels in congestive heart failure. AM. J. Med.**86**: 43-48.

Maggio, J.E. (1988). Tachykinins. Ann.Rev. of Neuroscience.**11**: 13-28.

Malayan, S.A., Ramsy, D.J., Keil, L.C. and Reid, I.A. (1980). Effects of increases in plasma vasopressin concentration on plasma renin activity, blood pressure, heart rate and plasma corticosteroid concentration in conscious dogs. Endocrinology. **107**: 1899-1904.

Marin, L., Sokucu, S., Gunoz, H., Saner, G., Neyzi, O. and Zetterstrom, R. (1988). Salt and water homeostasis during oral rehydration therapy in neonates and young infants with acute diarrhoea. II Rehydration with a solution containing 90 mmol sodium per litre (ORS 90). Acta Paediatr-Scand.**77**: 37-41.

Matsuda, M., Aono, M. and Okuma, M. (1993). Centrally administered neuropeptide Y (NPY) inhibits gastric emptying and intestinal transit in the rat. Digestive diseases and Sciences. **38**: 845-850.

Matzen, S., Schifter, S., Radvansky, J., Knigge, U., Warberg, J. and Secher, N.H. (1991). CGRP and leg vascular resistance during hypovolemic shock induced by head-up tilt in man. Acta Physiol. Scand. **142**: 313-318.

McKinley, M.J., Denton, D.A., Park, R.G. and Weisinger, R.S. (1983). Cerebral involvement in dehydration induced natriuresis. Brain Research.**263**: 340-343.

McKinley, M.J. and Oldfield, B.J. (1990). Circumventricular organs. The human nervous system. 415-438.

Metzler, C.H., Thrasher, T.N., Keil, L.C. and Ramsay, D.J. (1986). Endocrine mechanisms regulating sodium excretion during water deprivation in dogs. Am. J. Physiol. **251**: 560-568.

Modin, A., Pernow, J. and Lundberg, J.M. (1993). Neuropeptide Y and differential sympathetic control of blood flow and capacitance function in the pig and dog. Acta Physiol. Scand.**147**: 15-25.

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.

Moncada, S., Palmer, R.M.J. and Higgs, E.A. (1991). Nitric Oxide: Physiology, pathophysiology, and pharmacology. Pharmacological Reviews. **43**: 109-140.

Morris, H.R. (1984). Isolation and characterization of human CGRP.Nature.**313** : 746-748.

Morsing, P., Steinberg, A., Casellas, D., Mimran, A., Muller, S., Torup, C., Holm, C. and Persson, A.E.G. (1992). Renal interstitial pressure and tubulo-glomerular feedback control in rats during infusion of atrial natriuretic peptide (ANP). Acta Physiol Scand. **146**: 393-398.

Nelson, M.T., et al (1990). Arterial dilatations in response to CGRP involve activation of K^+ channels. Nature.**19**(344): (6468) 770-3.

Ohlen, A., Persson, M.G., Lindbom, L., Gustafsson, L.E. and Hedqvist, P. (1990). Nerve-induced nonadrenergic vasoconstriction and vaodilation in skeletal muscle. Am. J. Physiol. **258**: 1334

Page, I.H. and Helmer, O.M. (1940). A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin activator. J.Exp. Med.**71**: 29-42.

Palmer, R.M.J., Ferrige, A.G. and Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature.**327**: 524-526.

Pappas T., Debas H.T., Walsh, J.H., Rivier, J. and Tache, Y. (1986). Calcitonin-gene-related peptide (CGRP) induced selective inhibition of gastric acid secretion in dogs. Am.J.Physiol.**250**: G127-33.

Pernow, B. (1983). Substance P. Pharmacological reviews.**35**: 85-139.

Pernow, J. (1989). Actions of constrictor (NPY and Endothelin) and dilator (SP, CGRP, VIP) peptides on pig splenic and human skeletal muscle arteries: involvement of the endothelium. Br. J. Pharmacol.**97**: 983-989.

Pernow, J., Lundberg, J.M., Kaijser, L., Hjemdahl, P., Theodorsson-Norheim, E., Martinsson, A. and Pernow, B. (1986). Plasma neuropeptide-like immunoreactivity and catecholamines during various degrees of sympathetic activation in man. Clin. physiol.**6**: 561-578.

Pernow, J., Saria, A. and Lundberg, J.M. (1986). Mechanism underlying pre- and post-junctional effects of neuropeptide Y in sympathetic vascular control. Acta Physiol.Scand.**126**: 239-249.

Persson, K., Garcia-Pascual, A. and Andersson, K.E. (1991). Difference in the actions of calcitonin-gene-related peptide (CGRP) on pig detrusor and vesical arterial smooth muscle. Acta.Physiol.Scand.**143**: 45-53.

Persson, M.G., Gustafsson, L.E., Wiklund, N.P., Hedqvist, P. and Moncada, S. (1990). Endogenous nitric oxide as a modulator of rabbit skeletal muscle microcirculation *in vivo*. Br. J. Pharmacol.**100**: 463-466.

Pieribone, V., Brodin, L., Dahlstrand, J., Friberg, K., Soderberg, C., Larhammar, D. and Hokfelt, T. (1991). Neuronal expression of peptide YY mRNA in medulla oblongata of rat. Abstract.

Prabhkar, N.R., Rundold, M., Yamamoto, Y., Lagercrantz, H., Cherniack, N.S. and von Euler, C. (1987). Role of vagal afferents in SP-induced respiratory responses in anaesthsized rabbits. Acta Physiol.Scand.**131**: 63-71.

Prigle, J.K. and Berthiaume, L.M. (1988). Hypernatremia in calves. J.Vet.Intern.Med. **2**(2): 66-70.

Ralevic, V., Mathie, R.T., Alexander, B. and Burnstock, G. (1991). N^G-Nitro-L-arginine methyl ester attenuates vasodilator responses to acetylcholine but enhances those to Na nitroprusside. J. Pharm. Pharmacol.**43**: 871-874.

Ramsy, D.J., Thrasher, T.N. and Keil, L.C. (1988). Neurohormonal influences on vasopressin levels. In Vasopressin.

Ramsy, D.J., Keil, L.C., Sharpe, M.C. and Shinsako, J. (1978). Angiotensin II increases vasopressin, ACTH, and 11-hydro-corticosteroid secretion. Am.J.Physiol. **234**: R66-R71.

Ratten, S., Gonnella, P. and Goyal, R.K. (1988). Inhibitory effect of CGRP and calcitonin on opossum oesophageal smooth muscle. Gastroenterology. **94**: 284-93.

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

Regoli, D., Dion, S., Rhaleb, N-E., Drapeau, G., D'Orleans-Juste, P. (1990). Vasoactive peptides and their receptors. Blood vessels.**27**: 137-145.

Rigel, D.F. (1988). Effects of neuropeptides on heart rate in dogs: Comparison of VIP, PHI, NPY, CGRP and NT. Am.J.Physiol.**24**(2): H311-H317.

Roberts, J.D., Polaner, D.M., Lang, P., Zapol, M. (1992). Inhaled NO in persistent hypertension of the newborn. Lancet. **340**: 818-819.

Robertson, G.L. and Atar, S. (1984). The interaction of blood osmolality and blood volume in regulating plasma AVP level in man. J.Clin. Endocrino. and Metab.

Robertson, G.L. (1985). Regulation of vasopressin secretion. In D.W. Seldin & Giebisch (eds) The kidney Vol II, Raven Press, New York, 869-884.

Rosenfeld, M.G., Mermond, J.J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., River, J., Vale, W.W. and Evans, R.M. (1983). Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature.**304**: 129-35.

Rosenzweig, A. and Seidman, C.E. (1991). Atrial natriuretic factor and related peptide hormones. Ann.Rev.Bioch. **60**: 229-55.

Rudehill, A., Olcen, M., Sollevi, A., Hamberger, B. (1987). Release of neuropeptide Y (NPY) upon haemorrhagic hypovolemia in relation to vasoconstrictor effects in the guinea-pig. Acta Physiol. Scand.**131**: 517-523.

Saavedra, J.M., Israel, A., Plunkett, L.M., Kurihara, M., Shigematsu, K. and Correa, F.M.A. (1986). Qualitative distribution of A-II binding sites in rat brain by autoradiogrphy.Peptides.**7**: 679-678.

Safwate, A., Kati-Coulibaly, S., Davicco, M.J. Giry, J. Barlet, J.P. (1991). Renin-aldosterone system and arginine vasopressin in diarrhoeic calves. Br.Vet.J..**147**: 533-7.

Sagnella, G.A., Markandu, N.D., Angeh, S. and Mac-Gregore, G.A. (1985). Effects of changes in dietary sodium intake and saline infusion on immunoreactive ANP in human plasma. Lancet.**2**: 1208-1211.

Sahlgren, B., Eklof, A-Ch. and Aperia, A. (1992). Regulation of glomerular angiotensin II receptor densities in reno-vascular hypertension: response to reduced sympathetic and vasopressin influence. Acta Physiol. Scand.**146**: 467-471.

Saito, A., Kimura, S., and Goto, K. (1986). CGRP as potential neurotransmitter in guinea-pig right atrium. Am.J.Physiol.**250**: H693-98.

Saito, Y., Nakao, K., Nishimura, K., Sugawara, A., Okumura, K.O., Sonoda, R., Toshihiko, B., Hirofumi, Y. and Imura, H. (1987). Clinical application of atrial natriuretic peptide in patients with congestive heart failure : beneficial effects on left ventricular function. Circulation.**76**(1): 115-124.

Samson, W.K. (1985). Atrial natriuretic peptide inhibits dehydration and haemorrhage-induced vasopressin release. Neuroendocrinology.**40**: 277-279.

Samuelsson, U.E. and Jernbeck, J. (1991). Calcitonin-gene-related peptide relaxes porcine arteries via one endothelium-dependent and one endothelium-independent mechanisms. Acta Physiol.Scand.**141**: 281-282.

Seifert, H., Sawchenko, P., Chesnut, J., Rivier, J., Vale, W., Pandol, S.J. (1985). Receptor for calcitonin-gene-related peptide binding to exocrine pancreas mediates biological actions. Am.J.Physiol.**249**: G147-51.

Share, L. (1988). Role of vasopressin in cardiovascular regulation. Physiol.Rev.**68**: 1248-1284.

Sheikh, S.P., Holst, J.J., Nielson, T.S., Kaigge, U., Warberg, J., Theodorsso-Norheim, E., Hokfelt, T., Lundberg, J.M. and Schwarts, T.W. (1988). Release of NPY in pig pancreas: dual parasympathetic and sympathetic regulation. Am. J. Physiol.**255**: G46-G54.

Simon-Oppermann, Ch., Eriksson, S., Gray, D.A., Simon, E. (1987). Gradient of arginine vasopressin concentration but not angiotensin II conc. between cerebrospinal fluid of anterior third ventricle and cisterna magna in dogs. Brain research.**424**: 163-168.

Simon-Oppermann, Ch., Gray, D.A., Szczepanska-Sadowska, E. and Simon, E. (1983). Vasopressin in blood and third ventricle CSF of dogs in chronic experiments. Am.J.Physiol.**245**: 541-R548.

Sjoquist, M., Lee, S.L., Hoglundhal, A.U., Isaksson, B. and Ulfendahl, H.R. (1991). V1 antagonists attenuate a vasopressin-independent ICV-stimulated natriuresis. Abstract.

Stajarne, P., Lacroix, J.S., Anggard, A. and Lundberg, J.M. (1991). Compartment analysis of vascular effects of neuropeptides and capsaicin in the pig nasal mucosa Acta Physiol.Scand.**141**: 335-342.

Standaert, D.G., Cachetto, D.F., Needleman, P. and Saper, C.B. (1987). Inhibition of the firing of vasopressin neurons by atriopeptin. Nature.**329**: 151-153.

Stasch, J.P., Hirth, C., Kazda, S. and Neuser, D. (1989). Endothelin stimulates the release of atrial natriuretic peptides *in vivo* and *in vitro*. Life Sci. **45**: 869-875.

Steenbergh, P.H., Hoppener, J.W.M., Zandberg, J., Van de Ven, W.J.M., Jansz, H.S. and Lips, C.I.M. (1984). Calcitonin-gene-related peptide coding sequence is conserved in the human genome and is expressed in medullary thyroid carcinoma. J.Clin. Endocrinol. and Metabolism.**59**: 358-60.

Steiner, M. and Phillips, M.I. (1988). Renal tubular receptors down-regulated by dehydration. Am.J.Physiol. **254**: 404-410.

Sternium, C. and Brecha, N. (1986). Immunocytochemical identification of islet cells and nerve fibres containing CGRP-like immunoreactivity in the rat pancreas. Gastroenterology.**90**: 1155-63.

Sudoh, T., Kangawa, K., Minamino, N. and Matsuo, H. (1988). A new natriuretic peptide in porcine brain. Nature.**332**(3): 78-81.

Svendsen, J.H., Sheikh, S.P., Jorgensen, J., Mikelsson, J.D., Paaske, W.P., Sijrsen, P. and Hamso, S. (1990). Effects of neuropeptide Y on regulation of blood flow rate in canine myocardium. Am.J. Physiol.**259**: 1709-1717.

Szczepaska-Sadowska, Gray, D., and Simmon-Opperman (1983). Vasopressin in blood and third ventricle CSF during dehydration, thirst and haemorrhage. Am.J.Physiol. **245**: R549-555.

Tache, Y., Pappas, T., Lauffenburger, M., Goto, Y., Walsh, J.H. and Debas, H. (1984). CGRP potent peripheral inhibitor of gastric acid secretion in rats and dogs. Gastroenterology.**87**: 344-349.

Takeda, K., Lehnert, H.M., Kim, J.K. and Schrier, R.W. (1988). AVP-induced Ca^{++} and contraction of rat glomerular mesangial cells. Am.J.Physiol.**255**(1): F142-F150.

Tang, J., Webber, R.J., Chang, D., Chang, J.K., Kiang, J. and Wei, E.T. (1984). Depressor and natriuretic activities of several atrial peptides. Regul.Pept.**9**: 53-59.

Tatemoto, K., Carlqvist, M. and Mutt, V. (1982). Neuropeptide Y, a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature.(Lond.) **296**: 659-660.

Theodoroson-Norheim, E., Hemsen, A., Brodin, E. and Lundberg, J.M. (1987). Sample handling techniques when analyzing regulatory peptides. Life Sci.**41**: 845-8.

Thullen, J.D. (1988). Management of hypernatremic dehydration due to insufficient lactation. Clin-Paediatr-(Phila).**27**(8): 370-2.

Tikkanen, I., Fyhrquist, F., Metsarinne, K., Leidenius, R. (1985). Plasma ANP in cardiac disease and during infusion in healthy volunteers. Lancet.**2**: 66-69.

Toda, N. and Okamura, T. (1990). Mechanism underlying the response to vasodilator nerve stimulation in isolated dog and monkey cerebral arteries. Am.J.Physiol. 1511-1517.

Trachtman, H., Barbour, R., Sturman, J.A., Finberg, L. (1988). Taurine and osmoregulation. Taurine is a cerebral osmoprotective molecule in chronic hypernatremic dehydration. Paediatr-Res.**23**(1): 35-9.

Trachtman, H., del Pizzo, R., Sturman, J.A., Huxtable, R.J., Finberg, L. (1988). Taurine and osmoregulation. II Administration of taurine analogues affords cerbral osmo-protection during chronic hypernatremic dehydration.A.J.Dis.Child.**142**:1194-8.

Trachtman, H., Futterweit, S., del Pizzo, R. (1992). Taurine and osmoregulation. IV Cerebral taurine transport is increased in rats with hypernatremic dehydration. Pediatr-Res. **32**(1): 118-24.

Tsutsumi, K. and Saavedra, J.M. (1991). Characterization and development of ANG II receptor subtypes (AT_1 and AT_2) in rat brain. Am.J.Physiol. **261**: 209-216.

Ullman, J.E., Hjelmquvist, H., Lundberg, J.M. and Rundgren, M. (1992). Tolerance to haemorrhage during vasopressin antagonism and/or captopril treatment in conscious sheep. Acta Physiol.Scand.**146**(4): 457-465.

Velasquez-Jones, L., Becerra, F.C., Faure, A., de Leon, M., Moreno, H., Maulen, I., Abraham-Jalil, A., Muraira, A. (1990). Clinical experience in Mexico with a new oral rehydration solution with lower osmolality. Clin-Ther. 12 Suppl A: 95-103.

Vila, J., Esplugues, J.V., Martinez-Cuesta, M.A., Martinez-Martinez, M.C., Aldasoro, M., Flor, B. and Lluch, S. (1990). N^G-Monomethyl-L-arginine and N^G-nitro L-arginine inhibit endothelium dependent relaxations in human isolated omental arteries. J. Pharm. Pharmacol. **43**: 869-70.

Wade, C.E. and Ramsy, D. (1983). Role of volume and osmolality in the control of vasopressin in dehydrated dogs. Neuroendocrinology.**37**: 349-353.

Walker, B.R., Childs, M.E. and Adams, E.M. (1988). Direct cardiac effects of vasopressin: role of V1 and V2 vasopressinergic receptors. Am.J.Physiol.**255**(1): 261-265.

Winther, J.E., Espersen, K., Kanstrup, L.L. and Christensen, N.J. (1992). Age-related changes of exercise induced plasma catecholamines and NPY responses in normal human subjects. Acta Physiol. Scand.**144**: 129-133.

Woods, R.L. and Johnston, C.I. (1983). Contribution of vasopressin to the maintenance of blood pressure during dehydration. Am.J.Physiol. **245**: F615-F621.

Xie, Q-W., Cho, H.G., Calaycay, J., Mumford, R.A., Swiderek, K.M., Lee, T.D., Ding, A., Troso, T. and Nathan, C. (1992). Cloning and characterization of inducible nitric oxide synthase from mouse macrophages. Science.**256**: 225-228.

Yndgaard S., et al (1991). CGRP during head-up tilt in man. Acta Physiol. Scand. **143**: 313-318.

DECLARATION

I, the undersigned, declare that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

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