PLASMA ATRIAL NATRIURETIC PEPTIDES IN THE HORSE AND GOAT WITH SPECIAL REFERENCE TO EXERCISING HORSES

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Academic Dissertation

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CONTENTS

1. Abstract

Atrial natriuretic peptides, the COOH-terminal ANP_{99-126} (ANP), and the NH_2-terminal $NT\text{-}ANP_{1-98}$ (NT-ANP), the two fragments of the atrial natriuretic propeptide ANP_{1-126} were studied. ANP is regarded as a physiological regulator because of its vascular and renal effects, and its plasma concentration has been observed to increase on any occasion that strains the heart. It is well known that physical activity accelerates cardiovascular function and upsets fluid balance. To increase knowledge of atrial peptides and their responses to exercise in the horse, a species characterized as an extreme animal athlete, exercise tests on a treadmill were carried out in 37 horses. In addition, since cyclic changes are known to exist in physiological phenomena, and the previous results on circadian changes obtained in human subjects and rats have been contradictory, circadian variation in ANP was examined in two quadruped species, the horse and the goat, both of which represent species in which postural effects on ANP were expected to be minor.

The results confirmed that atrial peptides are released in horses in response to exercise. Increasing heart rate and work intensity, as well as the volume expansion known to increase atrial pressure, together with the functioning of the muscular and respiratory pumps were shown to have a contributory influence on these responses. Of the endocrine factors, noradrenaline was correlated with ANP at the start of exercise and may contribute to release of atrial peptides in exercising horses, directly or indirectly. The role of other pressor hormones during submaximal exercise appeared, however, to be small. Exercise-induced responses in ANP were modulated by water balance and physical condition. Standardizing the test according to heart rate response, however, abolished the effect of physical condition. Taken together, these results agree well with the theory that atrial stretch is the main determinant of atrial peptide release during acute stimulation

The temporal patterns in plasma ANP and NT-ANP differed, most probably due to differences in their plasma half-lives. NT-ANP was shown to be more stable in plasma than was ANP and can thus be useful in horses as an indicator of changes in plasma atrial peptides.

After exercise, plasma concentrations of atrial peptides returned slowly to their pre-exercise levels, in contrast to changes reported previously in human subjects. The temporal patterns of ANP and NT-ANP indicate that their release remained stimulated even after completion of exercise. Blood volume expansion, the respiratory pump, and heart rate partially explained these changes; the role of vasoactive hormones seemed to be minor. Intensity of exercise and changes in water balance were observed to modulate recovery of atrial peptides.

In the horse and the goat, plasma ANP levels were individual, ranging at rest from 1 to 10 pmol/l. Basal plasma NT-ANP concentration was 45-fold higher than in ANP, being in horses from 200 to 300 pmol/l. Basal plasma concentrations of ANP and NT-ANP were affected neither by breed nor by physical condition, but in relation to gender, the lower plasma concentrations occured in stallions. Circadian changes in ANP in physically inactive animals were minor, providing no evidence for circadian variation in plasma ANP.

In conclusion, the present results imply that ANP is a factor involved in regulation of cardiovascular control and fluid balance during and after exercise in horses.

2. List of original publications

I Kokkonen U-M, Hackzell, M & Räsänen, L 1995. Plasma atrial natriuretic peptide in Standardbred and Finnhorse trotters during and after exercise. *Acta Physiol Scand* 154: 51-58.

II Kokkonen, U-M, Pösö, AR, Hyyppä, S, Huttunen, P & Leppäluoto, J. In press. Exercise-induced changes in atrial peptides in relation to other neuroendocrine responses and fluid balance in the horse. *J Vet Med A*.

III Kokkonen, U-M, Hyyppä, S & Pösö, AR 1999. Plasma atrial natriuretic peptide during and after repeated exercise under heat exposure. *Equine vet J Suppl* 30: 184-189.

IV Nyman, S, Kokkonen, U-M & Dahlborn, K 1998. Changes in the plasma concentrations of atrial natriuretic peptide in the exercising horses in relation to hydration status and exercise intensity. *Am J Vet Res* 59: 489-494.

V Kokkonen, U-M, Riskilä, P, Roihankorpi, M-T & Soveri, T 2001. Circadian variation of plasma atrial natriuretic peptide, cortisol and fluid balance in the goat. *Acta Physiol Scand* 171:1-8.

3. Abbreviations

ACTH	adrenocorticotropic hormone / adrenocorticotrophin / corticotrophin
ADH	antidiuretic hormone / AVP
ANF	atrial natriuretic factor / ANP
ANP	ANP_{99-126} / the COOH-terminus of the prohormone ANP_{1-126} / atrial natriuretic peptide / A-type natriuretic peptide / ANF
ANP_A	guanylate cyclase receptor mediating the effects of ANP and BNP
ANP_B	guanylate cyclase receptor mediating the effects of CNP
ANP_C	clearance receptor of atrial peptides
ANOVA	analysis of variance
AVP	arginine vasopressin / vasopressin / ADH
BNP	brain natriuretic peptide / B-type natriuretic peptide
bpm	beats per minute
CET	competition exercise test / 60-min exercise with varying velocities
cGMP	guanosine 3',5'-monophosphate
CNP	C-type natriuretic peptide
EDTA	ethylenediaminetetraacetic acid
EXP 1	40-min steady-state exercise test at 65-70% of HR_{max}
EXP 2	12-min steady state exercise test at 90% of HR_{max}
HPLC	high-performance liquid chromatography
HR_{max}	maximal heart rate
NT-ANP	NT-ANP_{1-98} , NH_2-terminus of the prohormone ANP_{1-126}
PCV	packed cell volume / haematocrit value
RIA	radioimmunoassay
SET	standardised exercise test / 8- or 6-min exercise test with graded velocities

4. Introduction

The electron-microscopic studies in the 1950s and in the 1960s showed that mammalian heart atria contained storage granules which morphologically resembled secretory granules in endocrine cells (Kisch 1956, Jamieson & Palade 1964). Later on, it was shown that the granularity varied in situations of altered water-electrolyte balance (de Bold 1979). The secretory function of the atria did not become apparent until 1981 when de Bold with his colleagues demonstrated that atrial homogenate induced a massive, rapid diuresis and natriuresis in rats (de Bold *et al.* 1981). Soon after these findings, the relationship between atrial granularity and the natriuretic factor was confirmed (de Bold 1982), and the structure of the first peptide of the natriuretic family, atrial natriuretic peptide (ANP), was characterized (Flynn *et al.* 1983). Further studies showed that ANP plays a physiological role in body fluid and cardiovascular homeostasis by eliciting natriuresis, diuresis, reduction in plasma volume and in blood pressure, and inhibition of the release and action of other hormones. Other natriuretic peptides closely related to ANP have been identified subsequently. Active research and new analytical methods, especially radioimmunological assays, introduced first in the1960s (Yalow 1960), and methods based on molecular and cellular biology have had a decisive effect on the development of our understanding of cardiac endocrinology. Consequently, today, it goes without saying that, besides the contractile function, the mammalian heart also possesses an endocrine role by releasing physiologically active compounds into the circulation.

Physical activity strains the heart by increasing the cardiac work load. This occurs as a part of cardiovascular readjustment in order to supply the increased metabolic demands during exertion. Some previous results have shown that physical activity raises plasma ANP concentration in horses (McKeever *et al.* 1991a, b) and in goats (Kokkonen 1992). Considering the physiological effects of exogenously administered ANP: reduction in heart rate, stroke volume, cardiac output and blood pressure, as well as modulation of capillary filtration capacity, it is quite possible that ANP contibutes to adaptive changes in cardiovascular stress, for example, through regulation of blood flow. Among the animal models of exercise, the horse represents a species that can be regarded as a native athlete. Its respiratory and circulatory functions, e.g., oxygen uptake, delivery, and supply, as well as blood composition,

are specialized for exercise, endowing the horse with an excellent aerobic performance capacity (Erickson 1993). These are phenomena distinguishing the horse from ”sedentary” species, such as ruminants (Hoppeler 1990), and also from humans. Despite the marked increase in understanding of equine exercise physiology during the past ten years, more systematical research is required for a full description of atrial peptides as well as their interaction with cardiovascular and endocrine systems in horses. Although the overall knowledge of the whole natriuretic peptide family at present is wide, in horses, identification of these peptides, their release mechanisms, metabolism, function, and physiological importance are still to a great extent unknown.

To increase knowledge of atrial peptides in the horse, the experiments for this study were begun in 1992 as part of a new approach to equine cardiac endocrinology, to investigate effects of physical stress on plasma ANP, the COOH-terminal ANP_{99-126}, and NT-ANP, the NH_2-terminal NT-ANP_{1-98}, which are the two cleavage products of the prohormone ANP_{1-126}, until now the only natriuretic peptide identified in the horse. These responses were further assessed in relation to physiological factors known or expected to be involved in release of atrial peptides. It was also of interest to determine basal plasma level of ANP in the horse and to compare it with that in another quadruped species, here the goat. Knowing that circadian rhythm affects plasma levels of many hormones, it was important to learn more about circadian changes in ANP. While most of the studies have described circadian changes in ANP in humans and other plantigrades, the present study shows these changes in two quadruped species, adapted to living permanantly in a horizontal posture. Contrary to humans, the greatest part of the blood volume in quadrupeds exists at the level of the heart or above it, but it is unclear whether this has any importance in regulation of atrial peptides in those species.

5. Review of the literature

5.1 Atrial natriuretic peptide

5.1.1 Cardiac peptide hormones and their function

Atrial natriuretic peptide (ANP, also called A-type natriuretic peptide, ANF, atrial natriuretic factor, atriopeptin, auriculin, cardiodilatin, and cardionatrin) and brain natriuretic peptide (BNP or B-type natriuretic peptide, named for the tissue in which it was first discovered) are two rather well-known cardiac natriuretic polypeptides in mammals (for references, see Ruskoaho 1992, Nakao *et al.* 1992). Contrary to ANP, which is released from the atria, BNP is synthesized and secreted mainly by the ventricle (Nakao *et al.* 1992). The C-type natriuretic peptide (CNP), a structurally homologous peptide with the A- and B-type peptides, is the third gene product of the natriuretic family, but its major sites of synthesis are in vascular endothelial cells and the central nervous system, and it has been considered a paracrine regulator rather than a cardiac peptide (Minamino *et al.* 1991, Ogawa *et al.* 1995). Another hypotensive peptide, adrenomedullin, initially isolated from human pheochromocytoma cells (Kitamura *et al.* 1993), is also considered nowadays a cardiac hormone. In addition, other cardiac hormones, such as the salmon cardiac hormone (Tervonen *et al.* 1998), have been recently discovered.

The main effects of ANP and BNP include natriuresis, diuresis, vasodilatation, reduction in blood pressure and in plasma volume, inhibition of biosynthesis, and release and action of other hormones, such as those of the renin-angiotensin-aldosterone system, and of endothelin and AVP (Atlas & Laragh 1987, Charles *et al.* 1990,1993, 1996, Rosenzweig & Seidman 1991, Ruskoaho *et al.* 1997). Most of the biological functions of natriuretic peptides are mediated by intracellular accumulation of guanosine 3',5'-monophosphate (cGMP) through the activation of a particulate guanylyl cyclase (Hamet *et al.* 1984).

5.1.2 Structure and biosynthesis

The structure of ANP has been elucidated following cloning and sequencing of the complementary DNA and genes encoding ANP. The ANP gene is organised into three exons separated by two introns (Nakao *et al.* 1992, Ruskoaho 1992).

Transcription of the gene yields a messenger RNA that encodes a precursor molecule, preproANP, which contains between 149 and 153 amino acids depending upon the species (for references, see Nakao *et al.* 1992 and Ruskoaho 1992, Richter *et al.* 1998). PreproANP is cleaved in the endoplasmic reticulum by endoprotease into a signal peptide and into the126-residue-containing prohormone ANP_{1-126}, also called proANP. ProANP remains uncleaved under transport and is stored in this inactive form in the atrial cardiocytes (Forssmann *et al*. 1983, 1984, Thibault *et al.* 1987, Richter *et al.* 1998). This has been regarded as an unique character of ANP, because several other peptide hormones are processed during transport and in secretory granules, and are stored as bioactive hormones (Ruskoaho 1992). Biosynthesis of BNP, a distinct gene product, resembles that of ANP with the exception that the storage form in the heart is the cleaved mature form (Nakao *et al.* 1992).The amino acid sequence of proANP, comprising a molecule with molecular weight approximately 13 000, is well conserved among mammals (Kangawa *et al.* 1985, Ruskoaho 1992). Upon appropriate stimulus, proANP is cleaved into the COOH-terminal peptide, ANP_{99-126}, and NH_2-terminal peptide, NT-ANP_{1-98}, also called NT-ANP (Thibault *et al.* 1985, Michener *et al.* 1986). This conversion occurs close to the myocyte itself and almost simultaneously with secretion, probably by a serine protease, although some other enzymes may be involved as well (Inagami *et al.* 1989, for references, see Ruskoaho 1992).

The biologically active ANP is a 28-amino acid peptide with a ring structure formed by an intramolecular disulfide linkage between cysteine residues due to post-translational modification, and has a molecular weight of approximately 3000 (Kangawa *et al.* 1985). Its amino acid sequence is highly conserved in mammalian species, except for a single amino acid residue at the 12^{th} position which is methionine in most mammalian species but isoleucine in the rat, mouse, and rabbit (Table 1). Richter *et al.* (1998) have reported the amino acid sequence of equine ANP (Table1). The amino acid sequence of goat ANP has not been determined; however, the results in other ruminants (Vlasuk *et al.* 1986, Charles *et al.* 1990, Yandle *et al.* 1991, Aitken *et al.* 1999) suggest that also the amino acid sequence of caprine ANP may be homologous with that in other mammalian species.

BNP is closely related to ANP, as it also contains a carboxyl terminus with a 17-residue ring formed by a disulfide bond. In contrast to ANP, BNP is less homologous across species (Maekawa *et al.* 1988, Kojima *et al.* 1989, Seilhamer *et al.* 1989, Ogawa *et al.* 1994, Aitken *et al.* 1999). The structure of BNP in horses and goats has not been documented. However, porcine BNP-26 immunoreactivity, but not that of human BNP-32, has been shown in the auricular cardiocytes in horses (Mifune *et al.* 1995).

Table 1. Comparison of the COOH- terminal ANP_{1-28} sequences between various species (Flynn *et al.* 1983, Forssmann *et al.* 1984, Kangawa and Matsuo 1984, Seidman *et al.* 1984, Oikawa *et al*. 1985, Ong *et al.* 1986, Vlasuk *et al.* 1986, Yandle *et al.* 1991, Richter *et al.* 1998, Aitken *et al.* 1999).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Human	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Met**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Dog	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Met**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Horse	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Met**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Sheep	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Met**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Cow	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Met**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Pig	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Met**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Rat	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Ile**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Mouse	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Ile**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly
Rabbit	Ser	Leu	Arg	Arg	Ser	Ser	Cys	Phe	Gly	Gly	Arg	**Ile**	Asp	Arg	Ile	Gly	Ala	Gln	Ser	Gly	Leu	Gly

S - S	**23**	**24**	**25**	**26**	**27**	**28**
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr
	Cys	Asn	Ser	Phe	Arg	Tyr

The mammalian NT-ANP contains 98 amino acids and is well conserved (Table 2). For example, the sequence in sheep shows 71%, 75%, and 79% homology with the sequences from humans, pigs, and cows, respectively (Aitken *et al.* 1999), and that of equine NT-ANP 80 to 90% with the sequences from humans, cows, rats and mice (Vlasuk *et al.* 1986, Richter *et al.* 1998). The most marked differences in the sequences are in amino acids from 1 to 70 (Table 2). The length of the N-terminal extension of BNP differs from that of ANP, consisting of 108, 106, 105, and 96 amino acids in humans, pigs, dogs, and rats, respectively (Seilhamer *et al.* 1989).

Table 2. Comparison of the NH_2-terminal ANP_{1-98} sequences between various species (Oikawa *et al*. 1985, Vlasuk *et al.* 1986, Richter *et al.* 1998).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Human	Asn	Pro	**Met**	Tyr	**Asn**	Ala	Val	Ser	Asn	Ala	Asp	Leu	Met	Asp	Phe	Lys	Asn	Leu	Leu	Asp	**His**	Leu
Dog	Asn	Pro	Val	Tyr	Gly	Ser	Val	Ser	Asn	Ala	Asp	Leu	**Leu**	Asp	Phe	Lys	Asn	Leu	Leu	Asp	Arg	Leu
Horse	Asn	Pro	Val	Tyr	Gly	Ser	Val	Ser	Asn	**Gly**	Asp	Leu	Met	Asp	Phe	Lys	Asn	Leu	Leu	Asp	Arg	Leu
Cow	Asn	Pro	Val	Tyr	Gly	Ser	Val	Ser	Asn	Ala	Asp	Leu	Met	Asp	Phe	Lys	Asn	Leu	Leu	Asp	Arg	Leu
Rat	Asn	Pro	Val	Tyr	Ser	Ala	Val	Ser	Asn	**Thr**	Asp	Leu	Met	Asp	Phe	Lys	Asn	Leu	Leu	Asp	**His**	Leu

	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Human	Glu	Glu	Lys	Met	Pro	Leu	Glu	Asp	Glu	Val	Val	Pro	Pro	Gln	Val	Leu	Ser	Asp	**Pro**	Asn	Glu	Glu
Dog	Glu	**Asp**	Lys	Met	Pro	Leu	Glu	Asp	Glu	**Ala**	**Glu**	**Ser**	Pro	Gln	**Ala**	Leu	Ser	Asp	Gln	Asn	**Ala**	Glu
Horse	Glu	Glu	Lys	Met	Pro	Leu	Glu	Asp	Glu	Val	**Met**	Pro	Pro	Gln	Val	Leu	Ser	Asp	Gln	**Ser**	Glu	Glu
Cow	Glu	**Asp**	Lys	Met	Pro	Leu	Glu	Asp	Glu	**Ala**	Val	Pro	**Ser**	Gln	Val	Leu	Ser	**Glu**	Gln	Asn	Glu	Glu
Rat	Glu	Glu	Lys	Met	Pro	Val	Glu	Asp	Glu	Val	**Met**	Pro	Pro	Gln	**Ala**	Leu	Ser	**Glu**	Gln	**Thr**	Asp	Glu

	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Human	Ala	Gly	Ala	Ala	Leu	Ser	Pro	Leu	Pro	Glu	Val	Pro	Pro	Trp	Thr	Gly	Glu	Val	**Ser**	Pro	Ala	Gln
Dog	Ala	Gly	Ala	Ala	Leu	Ser	Pro	Leu	Pro	Glu	Val	Pro	Pro	Trp	Thr	Gly	Glu	Val	**Ser**	Pro	Ala	Gln
Horse	**Glu**	**Arg**	Ala	Ala	Leu	Ser	Pro	Leu	Pro	Glu	Val	Pro	Pro	Trp	Thr	Gly	Glu	Val	Asn	Pro	Ala	Gln
Cow	Ala	Gly	Ala	**Pro**	Leu	Ser	Pro	Leu	**Ser**	Glu	**Met**	Pro	Pro	Trp	**Met**	Gly	Glu	Val	Asn	Pro	Ala	Gln
Rat	Ala	Gly	Ala	Ala	Leu	Ser	Ser	Leu	Pro	Glu	Val	Pro	Pro	Trp	Thr	Gly	Glu	Val	Asn	Pro	**Ser**	Gln

	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88
Human	Arg	Asp	Gly	Gly	Ala	Leu	Gly	Arg	Gly	Pro	Trp	Asp	Ser	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser
Dog	Arg	Asp	Gly	Gly	Ala	Leu	Gly	Arg	**Ser**	Pro	Trp	Asp	Ser	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser
Horse	Arg	Asp	Gly	Gly	Ala	Leu	Gly	Arg	Gly	**Ser**	Trp	Asp	Ser	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser
Cow	Arg	Glu	Gly	Gly	**Val**	Leu	Gly	Arg	Gly	Pro	Trp	**Glu**	Ser	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser
Rat	Arg	Asp	Gly	Gly	Ala	Leu	Gly	Arg	Gly	Pro	Trp	Asp	Pro	Ser	Asp	Arg	Ser	Ala	Leu	Leu	Lys	Ser

	89	90	91	92	93	94	95	96	97	98
Human	Lys	Leu	Arg	Ala	Leu	Leu	**Thr**	Ala	Pro	Arg
Dog	Lys	Leu	Arg	Ala	Leu	Leu	Ala	Ala	Pro	Arg
Horse	Lys	Leu	Arg	Ala	Leu	Leu	Ala	Ala	Pro	Arg
Cow	Lys	Leu	Arg	Ala	Leu	Leu	**Thr**	Ala	Pro	Arg
Rat	Lys	Leu	Arg	Ala	Leu	Leu	Ala	**Gly**	Pro	Arg

The structure of ANP in nonmammalian species, both COOH- and NH_2-termini, differ considerably from those in mammals (for references, see Ruskoaho 1992). In chicken heart, the major natriuretic petide is a BNP-type peptide (Akizuki *et al.* 1991).

5.1.3 Storage in cells and tissue distribution

Cardiocytes of various animal species contain osmophilic granules with a remarkable degree of ultrastructural and cytochemical similarities (Jamieson & Palade 1964, Tomisawa 1969, Cantin *et al.* 1979). Their role as cardiac natriuretic peptide-containing granules has been established in humans, in rodents (de Bold 1982, Flynn *et al.* 1983, Forssmann *et al.* 1984, Kangawa & Matsuo 1984, Kangawa *et al.* 1985),

and in domestic animals (Hinze *et al.* 1989, Mifune *et al.* 1991, 1995, 1996, Richter *et al.* 1998). Atrial granules have been found all over the sarcoplasma (Tomisawa 1969), but those containing ANP are located principally in the perinuclear area of the cells (Mifune *et al.* 1991, Ruskoaho 1992). Immunohistochemical and immunoelectron microscopical studies have shown differences in the distribution and average diameter of ANP immunoreactive granules, since their number and diameter seem to be smaller in the horse than in the pig and cattle (Mifune *et al.* 1991). Similar polymorphism has already been found in the early histological studies on atrial granularity (Jamieson & Palade1964, Tomisawa 1969). The functional significance of these differences remains unexplained.

In adult mammals, the ANP immunoreactive granules concentrate mainly in the cardiocytes of the atria, whereas in fetuses and newborns they have been found in both the atria and ventricles (for references, see Ruskoaho 1992). ANP-specific mRNA, and proANP- and ANP-like immunoreactivity have also been shown in many other tissues, e.g., aortic arch, lung, central nervous system, adrenal, corpus luteum, skin, kidney, gastrointestinal tract, thymus, chorioidea and ciliary body, pancreas, thyroid gland, spleen, testis, liver, salivary gland, lacrimal gland, and fowl salt gland (for references, see Ruskoaho 1992). However, the ANP levels in these tissues are unlikely to cause significant changes in plasma concentrations, because, in physiological conditions, they have been far lower than those in the atrial cells (for references, see Gutkowska & Nemer 1989). ANP-like peptides and their precursors have been described in hearts of nonmammalian species, but, in them, interspecies differences seem to be considerable (for references, see Ruskoaho 1992, Mifune *et al.*1996).

5.1.4 Release

ANP and BNP are released continuously from the heart, but the rate of the release increases in response to appropriate stimuli. The reviews by Ruskoaho (1992), de Bold *et al.* (1996), Ruskoaho *et al.* (1997), and Thibault *et al.* (1999) have described a number of factors associated with plasma ANP level or stimulation of ANP secretion from the heart. These include:

1. **STRETCHING OF MYOCYTES**
   volume expansion, sodium intake, water deprivation, haemorrhage, water immersion, posture
2. **INTRACELLULAR- AND ENDOTHELIUM–DERIVED FACTORS**
   cationic environment (Ca^{2+}), Na-K-ATPase inhibitors, endothelin, nitric oxide, prostaglandins
3. **HEART RATE**
4. **NEUROHUMORAL AND OTHER ENDOCRINE FACTORS**
   adrenaline, noradrenaline, acetylcholine, vasopressin, glucocorticoids, thyroid hormone, angiotensin II, opiates
5. **PERIPHERAL AND CENTRAL NERVOUS SYSTEM**
6. **OSMOLALITY**
7. **HYPOXIA AND HYPERCAPNIA**
8. **MYOCARDIAL ISCHEMIA AND METABOLIC CHANGES**
9. **OTHER FACTORS**
   age, circadian variation, exercise, heat exposure, cold

Regulation of the release of atrial peptides seems to be complex, since experimental results thus far imply that endocrinologically the heart responds to different haemodynamic challences in acute, subacute, and chronic conditions with specific changes in transcription, translation, post-translational processing, storage, and release of ANP and BNP (de Bold *et al.* 1996). Although the principles of the release are fairly well known nowadays, the exact cellular mechanism resulting in augmentation of the release of ANP and BNP has not been settled.

Myocyte stretch is regarded as the central regulator of ANP and BNP secretion (Ruskoaho 1992, de Bold *et al.* 1996, Ruskoaho *et al.* 1997). Recent results have provided evidence that the mechanisms controlling ANP release may, however, be more complex, and the action of wall stretch may also occur via endogenous paracrine and autocrine vasoactive factors such as endothelin-1, nitric oxide, and angiotensin II (Ruskoaho *et al.* 1997).

Earlier experiments *in vivo* and *in vitro* have provided evidence that tachycardia stimulates ANP secretion from the heart (for references, see Ruskoaho 1992), but there are also experimental findings opposing its role as a primary stimulator of ANP. These include, for example, the fact that increased heart rate *in vivo* is generally accompanied by haemodynamic changes which may explain the ANP release. Further, some studies have shown that pacing the heart does not increase ANP release, and that ANP levels can be increased under experimental conditions in

which atrial tension develops concomitantly with inhibition of beating (for references, see Ruskoaho 1992, de Bold *et al.* 1996).

5.1.5 Circulating peptides and their biological activity)

Results of studies *in vitro* and *in vivo* have shown that ANP and NT-ANP are co-secreted and released in equimolar amounts into the circulation (Itoh *et al.* 1988, for references, see Ruskoaho 1992). The major circulating peptides derived from $proANP_{1-126}$ are NT-ANP_{1-98} and ANP_{99-126}. ANP is the biologically active hormone, whereas it is widely accepted that the NH_2-terminus of ANP does not show biological activity (for references, see Ruskoaho 1992). However, some studies have suggested that smaller fragments derived from human NT-ANP circulate and have diuretic and natriuretic effects, for example $proANP_{31-67}$ in humans (Vesely *et al.* 1994), rats (Martin *et al.* 1990), dogs (Habibullah *et al.* 1995), and monkeys (Benjamin & Peterson 1995). The results of the laboratory that first isolated and chemically characterised the fragments of $proANP_{1-126}$ do not support this latter view (Weir *et al.* 1994).

5.1.6 Receptors of natriuretic peptides

Specific ANP-binding sites have been reported in various organs such as endothelial cells, vascular smooth muscle cells, inner medullary collecting duct cells, and in organs such as the lung, kidney, adrenal gland, liver, and intestine (for reviews, see Gerbes & Vollmar 1990, Maack 1992). Three different receptor isoforms exist for the natriuretic peptides ANP_A (GC_A, NPR-A or NP-A), ANP_B (GC_B, NPR-B or NP-B), and ANP_C (NPR-C). The ANP_A and ANP_B receptors are single transmembrane types and are particulate guanylate cyclases (Inagami *et al.* 1995). ANP_A receptors are important in mediating the physiological effects of ANP and BNP, whereas CNP acts via ANP_B receptors to affect vascular cell growth and remodelling (Inagami *et al.* 1995). The results of Leskinen *et al.* (1997) suggest further that ANP may modulate its own release by ANP_A receptors *in vivo*. ANP_C receptors, which are not gualylate cyclases, are responsible for clearance of atrial peptides from the circulation, and, in this manner, play a role in the homeostasis of the circulating peptide level (Almeida *et al.* 1989, Suga *et al.* 1992). The binding affinity of cardiac peptides to ANP_C receptors, reported in humans and rats, is as follows: ANP>CNP>BNP (Suga *et al.* 1992). In kidney (glomeruli), lung membranes, adrenal zona glomerulosa cells, and

bovine aorta endothelial cells, the majority of ANP receptors seems to be ANP_C receptors (Gerbes & Vollmar 1990). Evidence exists that manipulations that alter blood volume and pressure also alter plasma ANP levels and ANP_C receptor density. Many findings support the theory that a decreased number of ANP_c binding sites results in an increase in plasma ANP concentration, and in a relatively greater amount of ANP binding to ANP_A receptors (for references, see Ruskoaho 1992).

5.1.7 Metabolism

Receptor-mediated binding, uptake, and metabolism in target tissues, degradation by enzymes and other processes at plasma membranes, and excretion, for example, into urine may be involved in ANP clearance from the circulation (Ruskoaho 1992). The major mechanisms for ANP clearance are uptake by clearance receptors and enzymatic degradation by neutral endopeptidase (endopeptidase EC 3.4.24.11, also called encephalinase and atriopeptidase) or some other enzymes (Gerbes & Vollmar 1990, Ruskoaho 1992). Various organs such as the lung, liver, intestine, and kidney, as well as the limbs, contribute to clearance of atrial peptides from the circulation (Hollister *et al.* 1989, Gerbes & Vollmar 1990). A general arterio-venous organ extraction ratio for ANP has been reported to be about 35%. The blood flow in different organs is variable and determines the contribution of these organs to the total body clearance of atrial peptides (Gerbes & Vollmar 1990). For NT-ANP, the kidney may be the major site of degradation (Katsube *et al.* 1986, Itoh *et al.* 1988, Sundsfjord *et al.* 1988).

Disappearance of ANP from the circulation consists of fast and slow components, the former of which accounts for up to 90% of the removal (Nakao *et al* 1986, Yandle *et al.* 1986). The half-life of ANP at rest is reportedly 16 s to 2.5 min in rats, 1.2 to 4.7 min in humans, and 3 to 4 min in sheep (Nakao *et al.* 1986, for other references, see Scarborough 1989 and Ruskoaho 1992, Charles *et al.* 1996). The half-life of NT-ANP is about 8- to 10-fold longer than that of ANP (Katsube *et al*. 1986, Ruskoaho 1992). The pharmacokinetics of atrial peptides has not been reported in horses and goats. Ageing has been reported to prolong the the slow-phase disappearance of ANP in human beings (Ohashi *et al.* 1987). Despite the rapid half-life of ANP in plasma, its degradation in blood *in vitro* is reported to be considerably slower (Scarborough 1989).

5.1.8 Plasma levels

Basal plasma ANP levels of 3 to 8 pmol/l have been reported in horses (McKeever *et al.* 1991a, b, 1992a, McKeever & Malinowski 1999), 2 to 5 pmol/l in 30-day-old Jersey calves (Wolf *et al.* 1991), 5 to 21 pmol/l in goats (Olsson *et al.* 1989, Kokkonen 1992), 5 to 25 pmol/l in humans (Fyhrquist *et al.* 1987, Freund *et al.* 1988, Itoh *et al.* 1988, Leppäluoto & Ruskoaho 1990, Baker *et al.* 1991, Follenius *et al.* 1992, Cugini *et al.* 1992, Kool *et al.* 1994, Nose *et al*. 1994, Arjamaa *et al.* 1996), 7 to 29 pmol/l in sheep (Charles *et al.* 1990, 1993), and 11 to 45 pmol/l in dogs (*Goetz et al.* 1986, Metzler *et al.* 1986, Geer *et al.* 1988, Hinze *et al.* 1989, Mäntymaa *et al.* 1994). In rats, the level has been variously from 20 to 150 pmol/l (Kanervo *et al.* 1991, Oliveira *et al.* 1993 Leskinen *et al.* 1994, Mäntymaa *et al.* 1994).

Plasma NT-ANP levels in humans and rats are 10- to 50-fold higher than those of ANP (Sundsfjord *et al.* 1988, Buckley *et al.* 1989, Baker *et al.* 1991, Vuolteenaho *et al.* 1992, Leskinen *et al.* 1994, Arjamaa *et al.* 1996, Wijbenga *et al.* 1999). The difference in basal plasma levels has been explained by a difference in these peptides' plasma clearance (Ruskoaho 1992). Because of a more stable plasma concentration, NT-ANP has been regarded as a good alternative means to measure plasma ANP release, especially for diagnostic and prognostic purposes in human medicine (Kettunen *et al.* 1994).

Results concerning the effect of age on plasma ANP are controversial. Ageing has been proposed to increase ANP levels, since, in humans and rats, higher plasma ANP levels occur in the elderly (Cugini *et al.* 1992, Wu *et al.* 1997). Contrary to these results, however, McKeever & Malinowski (1999) observed no difference in resting plasma ANP concentrations between old (22 years old) and young (5 years old) horses. Clark *et al.* (1990) show in their study that plasma ANP concentrations are higher in young women (19-42 years) than in young men (19-45). However, ANP levels in postmenopausal women (64-80) were not greater than in elderly men (62-86). Decreased ANP content has been reported in the atrial and hypothalamic tissue of old rats (Wu *et al.* 1997).

Elevated plasma natriuretic peptide levels have been associated with various diseases involving a pressure and/or fluid overload of the heart (Tikkanen *et al.* 1985, Fyhrquist *et al.* 1987, Kettunen *et al.* 1994, Häggström *et al.* 1997, Pouta *et al.* 1997), and in humans they have been evaluated as prognostic indicators in risk stratification and as markers of impaired cardiac function (Omland *et al.* 1996, Muders *et al.* 1997). Clinical applications of natriuretic peptides for veterinary medicine have been less well documented.

5.1.9 Circadian rhythm

Circadian rhythm represents a 24-h biological rhythm related to external or internal cycles, such as daily light-dark cycle, changes in day length or the rest-activity cycle (Wollnik 1989). Circadian changes in many physiological variables are well known. Some studies have reported circadian rhythm in plasma ANP in humans (Donckier *et al.* 1986, Winters *et al.* 1988, Bell *et al.* 1990, Vesely *et al.* 1990, Cugini *et al.* 1992, Sothern *et al*. 1996) and rats (Kanervo *et al.* 1991, Oliveira *et al.* 1993), whereas others have found in humans no circadian fluctuations (Richards *et al.* 1987, Follenius *et al.* 1992, Chiang *et al.* 1994, Kool *et al.* 1994). The report by Wolf *et al.* (1991) showed in calves two daily increases in plasma ANP, but these were possibly due to feeding or to alteration in plasma volume. Quadrupeds are useful subjects for investigating circadian changes, because the effects of postural changes, which may complicate the studies in humans (Gillies *et al.* 1987), do not affect the interpretation.

5.2 Exercising horse

5.2.1 Horse breeds and interbreed differences

Modern horse breeds are derived from different ancestors and comprise three main types, warm-blooded, half-blooded, and cold-blooded horses, named after the amount of their heredity from the warm-blooded Arab or Thoroughbred horses. For example, Standardbred trotters, having their origin in Thoroughbreds, represent warm-booded horses, which are typically graceful and fast. Half-bred horses are crosses between Thoroughbreds and heavy horses or native ponies. Cold-blooded horses are derived from the heavy pre-historic horse of central Europe and are stocky and slow in movement. The Finnhorse represents an old general type of horse (regarded as a cold-blooded horse), which was earlier used as a draught horse but which is nowadays used mainly for trotting and riding. It differs from the

Standardbred, for example, in haematological variables, muscle fibre composition, and metabolic response to exercise (Pösö *et al.* 1983, 1987, Räsänen *et al.* 1993). Whether or not interbreed differences exist in plasma atrial peptides remains unknown.

5.2.2 Types of exercise and exercise testing

As prey animals, the ability of horses to fast and to endure exercise performance has been essential. With domestication, these features have not been lost and have even been improved, for example, by development of new breeds such as the Thoroughbred.

In horse races, horses utilize their characteristic behaviour in running distances which vary from sprint to endurance. In trotting races the distances are usually from 1600 to 3100 m and require from 2 to 5 minutes. A 3-day event has been regarded as the most demanding competition for riding horses. It consists of three distinct parts, one of which is the endurance ride of approximately 35 km, consisting of different phases with varying velocities, from trot to gallop.

In the past, equine exercise physiology was studied principally in connection with competitions on a racetrack, but today, treadmill exercise is most commonly used. As compared to earlier racetrack tests, exercise tests on a treadmill allow more accurate standardization of the experiments, e.g., in relation to work intensity and environmental conditions, and make possible different kinds of sampling during exercise (Persson 1983, Sexton & Erickson 1990, Sheerman & Morris1990, Marlin *et al.* 1994). Moreover, the much greater exercise capacity of horses than of many other species, for example humans, as well as wide differences in experimental design, often limit direct comparisons between results for different species. Therefore, only a few comparisons with other species will be discussed.

5.2.3 Cardiovascular responses

The cardiovascular system responds to exercise with a rapid chronotropic and inotropic effect, characterised by increases in heart rate, cardiac contractility and shortening of systole. These are controlled by the autonomic nervous system, which exerts its effect through a local release of catecholamines from the sympathetic

nerves or through their release from the adrenal medulla. Increases in heart rate and contractility of the heart increase cardiac output (Stephenson 1997), which in horses can achieve a level 2- to 3-fold larger than for ponies and other species (Fregin & Thomas 1983, Hoppeler 1990, Hopper *et al.* 1991). Stroke volume increases as a result of increases in cardiac contractility (Stephenson 1997) as well as in blood volume (Hopper *et al.* 1991). At high-intensity, works such as graded exercise to maximal work intensity (Hopper *et al.* 1991, McKeever *et al.* 1993b) and competition exercise test (CET) (Marlin *et al.* 1996), which is an equine endurance test consisting of several exercise phases including two high-intensity phases, cardiac output, right atrial pressure, and stroke volume have been reported to be elevated. In contrast, low-intensity steady-state exercise, which increases heart rate and cardiac output, does not alter stroke volume and right atrial pressure significantly (Hinchcliff *et al.* 1990). Systemic arterial pressure remains constant during low-intensity exercise, but during more strenous exercise it increases, irrespective of any reduction in total peripheral resistance (Hinchcliff *et al.* 1990, Hopper *et al.* 1991, Erickson 1993, McKeever *et al.* 1993b). Finally, McKeever *et al.* (1993a, b) in splenectomised and intact horses provide evidence that the spleen plays an important role in raising right atrial pressure as well as plasma ANP concentration during exercise.

Heart rate varies in horses from the basal level of 35 bpm up to as high as 250 bpm. Elevation in heart rate occurs individually, and is linear and related to work intensity between the heart rate levels of 120 to 210 bpm (Persson 1983, Sexton & Erickson 1990, Hopper *et al.* 1991, McKeever *et al.* 1991a, 1993b, Marlin *et al.* 1996). At rates below 120 bpm, parasympathetic withdrawal may affect the changes (Hamlin *et al.* 1972). If exercise is continued at a constant work intensity in thermoneutral conditions, the heart rate remains relatively stable after an initial increase (Lindholm & Saltin 1974, Hinchcliff *et al.* 1990, Geor *et al.* 1995). Exposure to exceptional ambient conditions may affect such cardiovascular responses to exercise as heart rate, which increases in relation to a rise in ambient temperature and relative humidity until physiological adaptation has occurred (Geor *et al.* 1995, 1996).

Total peripheral resistance decreases during exercise (Hinchcliff *et al*. 1990, Hopper *et al.* 1991), indicating that a substantial portion of the body's skeletal muscle is involved in exercise (Stephenson 1997). Neural factors, hypoxia, and local

vasoactive metabolites released by contracting muscles all elicit vasodilatation and increased blood flow to exercising muscles (Stephenson 1997) and to the skin, when ambient temperature and relative humidity are increased (Erickson 1993). The study by Parks and Manohar (1983) showed in ponies a 70- to 76-fold increase in blood flow to muscles in the limbs during severe exercise, but a reduction of 19% from the resting values in renal blood flow. It has been experimentally shown that ANP has vasorelaxant effects (Atlas-Laragh 1987), and its plasma concentration increases in response to exercise. Thus, it has been speculated that ANP may contribute to modulation of haemodynamic changes during exercise.

5.2.4 Blood volume expansion

In horses, exercise produces a marked blood volume expansion, due to contraction of the spleen (Persson 1967, Persson *et al.* 1973). This occurs rapidly at the onset of exercise (Persson 1967, McKeever *et al.* 1993a) under the influence of the sympathetic nervous system and circulating catecholamines (Davies & Withrington 1973, Ericson 1993). The amount of released blood is dependent on work intensity, age, sex, and training state, and may be as high as 12 l (Persson 1967, Persson *et al.* 1973). The high packed cell volume (PCV) in the splenic blood is related to a high increase in venous PCV , which in horses may reach 15% (Persson 1967, Hopper *et al.* 1991, McKeever *et al.* 1993a). This is considerably higher than the about 3 to 4% reported in human beings (Green *et al*. 1984, Laub *et al.* 1993). Blood volume expansion together with muscle movements and increased intrathoracic pressure, supplements venous return and increases atrial and myocardial fibre stretch and pressure (Hopper *et al.* 1991, Erickson 1993), factors involved in release of cardiac hormones. In addition, in quadrupeds, about 70% of blood volume is at or above the level of the heart (Melbin & Detweiler 1993), whereas in man with an upright posture this same amount is below heart level, a fact that may have some importance for mechanisms related to cardiovascular homeostasis in an exercising quadruped.

5.2.5 Hormonal responses

Exercise increases plasma ANP level in humans (Freund *et al.* 1988, Vollmer- Larsen *et al.* 1989), dogs (Miller *et al.* 1990, Mäntymaa *et al.* 1994), rats (Ruskoaho *et al.* 1989a, Fareh *et al.* 1992, Mäntymaa *et al.* 1994), and in horses (McKeever *et al.* 1991a, b, McKeever & Malinowski 1999). Considering overall knowledge on atrial

peptides, data in horses have been restricted to only a few investigations. McKeever *et al.* (1991a, b) have reported an increase in plasma ANP, which was 4-fold during endurance exercise at the intensity of 55 to 60% of the HR_{max} and 6- to 7-fold at maximal work intensity. In their study (1991a), plasma ANP rose linearly and proportionally to $\%HR_{max}$. McKeever *et al.* (1991a) also reported on a correlation between ANP and increase in heart rate. Another study by McKeever *et al.* (1991b) shows that steady-state submaximal exercise is associated with increases in urine flow and sodium excretion, as well as in an increase in the plasma ANP concentration. In horses, the magnitude of the ANP response to exercise has been reported to be ageing-related (McKeever & Malinowski 1999).

Exercise increases plasma levels of many hormones, such as ACTH (Church *et al.* 1987, McCarthy *et al.* 1991), β-endorphin (McCarthy *et al.* 1991, Erickson 1993), arginine vasopressin (AVP)(McKeever *et al.* 1991b, 1992b), cortisol *(*Church *et al.* 1987, Erickson 1993), catecholamines (Snow *et al.* 1992), and the hormones of the renin-angiotensin-aldosterone system (McKeever *et al.* 1991a, 1992b). These are hormones involved in the control of cardiovascular function and fluid balance as well as with stress and pain relief. Plasma catecholamine concentrations reflect the response of the sympathetic nervous system. It is still confusing as to whether these hormones interact with atrial peptides in exercising horses. In addition, a variety of factors such as mode of exercise, hydration status, training status, diet, environmental factors, and posture may modulate hormonal responses to exercise *(Wade et al.* 1989), but regarding ANP, these relationships are less well known in horses.

5.2.6 Thermoregulation and water balance

Heat is dissipated by conduction, convection, radiation, and evaporation. Increased cardiac output as well as redistribution of blood flow promote heat transfer to the body core and its loss to the environment (Erickson 1993). In horses and humans, differently from other species, the primary mechanism of heat loss is evaporation of sweat (Carlson 1983), but the respiratory tract also contributes to heat and water loss (Erickson 1993, Hodgson *et al.* 1993). Sweating is controlled by both circulating adrenaline and the sympathetic nervous system, but during heat exposure only the latter is involved (Snow 1977, Carlson 1983). In a hot environment, sweating is an important mechanism for heat evaporation and may lead to fluid losses of 10 to

15 l/h, and to hypovolemia and dehydration (Carlson 1983, Hodgson *et al.* 1993, McCutcheon *et al.* 1995). When the ambient humidity is very high, the vapour pressure gradient between the body surface and the environment is less, and evaporation does not occur completely (Carlson 1983, McCutcheon *et al.* 1995). When the maximum rate via sweating is exceeded, heat loss via respiration may acts as a compensatory mechanism (McConaghy *et al.* 1996) and may reach 15 to 25% of the heat loss (Guthrie & Lund 1998). Many results suggest that heat exposure, which can cause significant alterations in fluid balance and cardiovascular function, can also lead to adaptive changes that attenuate thermal stress and improve thermal tolerance (Art *et al.* 1995**,** Geor *et al.* 1995, 1996, Harris *et al.* 1995, Lindinger *et al.* 1995, Hyyppä *et al.* 1996, Marlin *et al.* 1996).

Loss of water and electrolytes through fluid shifts and sweating is the principal means of reduction in plasma volume caused by exercise (Carlson 1983). In horses, this decrease is reportedly from 5 to10% following a short submaximal graded exercise (Persson 1967, McKeever *et al.* 1993a). ANP is suggested to be involved in fluid shifts between the intravascular space and interstitium under resting conditions (Olsson *et al.* 1994) and during exercise (Nagashima *et al.* 1995).

5.2.7 Training

Many studies describe in horses physiological responses to training, but a wide difference in experimental design often limits interpretation of results. In general, maximum oxygen uptake, blood and plasma volumes, and PCV and other blood cell variables increase with training, whereas the slope of regression of the submaximal heart rate on work intensity is less steep (Persson 1967, Rose and Hodgson 1982, Persson 1983, McKeever *et al.* 1987, Rose & Evans 1987, Knight *et al.* 1991). Training also results in greater sweat sensitivity but a decrease in total sweat fluid and in ion losses (McCutcheon & Geor 1996). Few studies have specifically addressed the issue of endocrine responses and exercise training in horses.

5.2.8 Apprehension and anxiety

Psychogenic factors, such as excitement, anxiety, and fear, result in activation of the sympathetic nervous system and release of catecholamines which on the other hand induce cardiovascular and haematological changes (Stewart *et al.* 1977, Rose &

Hodgson 1982, Revington 1983, Erickson 1993). Revington (1983) has shown in Thoroughbreds, for example, significant effects of pre-race excitement on the haemogram before competition. Whether these psychogenic factors have an effect on plasma ANP concentration has not been studied in horses.

5.2.9 Recovery

Heart rate decreases sharply within the first minutes after completion of work effort, but may remain slightly elevated for some time afterwards (Sexton & Erickson 1990, Geor *et al*. 1995). The return is dependent on duration and intensity of work effort, physical working capacity, and on ambient temperature and humidity (Persson 1967, Carlson 1983, Geor *et al.* 1995). The level of post-exercise activity may affect recovery. A report by Lovell and Rose (1995) showed that the initial decrease in heart rate was faster when the horses stood still after exercise than when they were walking for the same length of time, but 20 min after exercise the changes were similar, irrespective of level of post-exercise activity. Clearance of lactate from skeletal muscle was more rapid when the horses walked after exercise. Re-accumulation in the spleen has been reported to be slower than emptying and may require even more than 1 h if the spleen has been maximally emptied (Persson 1967). Relatively less information is available on the changes during return after exercise in horses. None of the previous studies in horses have described post-exercise changes in cardiac peptides.

6. Aims

The aims of this research were:

1) to determine basal plasma atrial natriuretic peptide levels in the horse and in the goat, and to assess them in relation to species, breed, sex, age, and physical condition

2) to study in horses the effects of physical activity on plasma ANP and NT-ANP concentrations during and after exercise

3) to study exercise-induced changes in plasma atrial peptides in relation to exercise type and factors associated with their plasma levels or secretion

4) to determine circadian changes in ANP and fluid balance

7. Materials and methods

7.1. Animals

The animals used were 4 Standardbred horses from the Faculty of Veterinary Medicine at the Swedish University of Agricultural Sciences, Uppsala, Sweden (Study IV), and 8 Finnhorses from the Agricultural Research Centre, Equine Research, Ypäjä, Finland (Studies II, III). The rest of the horses, 12 Finnhorses, 7 Standardbreds, and 6 half-bred riding horses, were owned by private persons (Studies I, III). The 10 goats were from the Faculty of Veterinary Medicine at the University of Helsinki, Finland (previously College of Veterinary Medicine) (Study V).

Table 3. Information on animals from original Studies I-V.

Species	Breed/Race	Number of animals	Females	Males	Age (years ± SD)	Weight (kg ± SD)	Number of the original study
Horse	Finnhorse	20[a]	9	11[b]	8.7 ± 2.4	522 ± 46.1	I, II, III
	Standardbred	11	4	7[c]	6.7 ± 3.3	467 ± 50.9	I, IV
	Half-bred	6	2	4[d]	9.5 ± 2.2	539 ± 88.9	III
Goat	Finnish landrace	10	10	-	6.4 ± 3.1	44.8 ± 8.3	V

[a] 3 horses used in two experiments; [b] 7 geldings; [c] 5 geldings; [d] 4 geldings

All horses were subjected to regular training, and most of them were in condition to perform in an actual race or 3-day event. The horses were accustomed before the experiment to running on a treadmill. All the goats were well accustomed to customary experimental procedures, such as being handled, catheterized, and sampled, and being restrained while standing in metabolism cages.

Subjecting the animals to any painful stimuli and extra restraint was carefully avoided to minimize the effect of emotional stress. The experimental designs were approved by the Ethics Committee for Animal Experiments of the College of Veterinary Medicine and the National Veterinary and Food Research Institute, the Ethics Committee for Animal Experiments of the Agricultural Research Centre, and the Uppsala Local Ethics Committee.

7.2 Experimental procedures

7.2.1. Exercise tests

7.2.1.1 Submaximal graded exercise test (Standardised Exercise Test = SET; Studies I, II)

SET was performed on a treadmill with a 3.5° (=6.3%) incline at the Faculty of Veterinary Medicine of the University of Helsinki. The seven Standardbreds performed the exercise intervals at speeds of 6, 7, 8, and 9 m/s, and the Finnhorses at speeds of 4, 5, 6, and 7 m/s (n=8) or 5, 6, 7, and 8 m/s (n=6). The four Finnhorses performed only three exercise intervals at speeds of 5, 6, and 7 m/s (Study I). These exercise levels were determined in a preliminary test so that the heart rate of each horse was ~ 200 bpm at the highest treadmill speed. A steady-state heart rate was reached within each exercise period.

7.2.1.2 Endurance exercise test with varying velocities (Competition Exercise Test = CET; Study III)

CET, designed to simulate the endurance test of a 3-day event, was carried out at the Equine Research Centre in Ypäjä, Finland. Six horses, trained at a mean ambient temperature of 7 ± 6.7 °C before and during the experiment, performed the test on a treadmill at a mean temperature of 28 ºC and relative humidity of 58%. CET was repeated 5 times at 2-week intervals (*Trials 1-5*), with the exercise always beginning at the same time of the day. The test included the following phases: a 10-min walk (1.7 m/s), 10-min trot (Phase A, 3.7 m/s), 2-min gallop (Phase B, 10.0 m/s), 20-min trot (3.7 m/s), and 10-min walk (1.7 m/s) (Phase X), 8-min canter (Phase D, 8 m/s), and a 30-min walk (active recovery, 1.7 m/s). The incline of the treadmill was 3º except for the walk during recovery, which was performed on a flat surface.

7.2.1.3 Submaximal steady-state exercise tests (Study IV)

Two steady-state exercise tests, EXP 1and 2 EXP 2, were carried out at the Faculty of Veterinary Medicine of the Swedish University of Agricultural Sciences in Uppsala, Sweden. In EXP 1, four Standardbreds exercised on a flat surface at a constant treadmill speed eliciting 65 to 70% of maximal heart rate (HR_{max}). The horses were studied during normohydration (ad libitum access to water), dehydration (water withheld for 24 h before exercise), and hyperhydration (12 l of tapwater at body

temperature through a nasogastric tube during a 10-min period 30 min before entering the treadmill). In EXP 2, the same horses performed an exercise test during normohydration at a 3.5° incline at a constant treadmill speed eliciting 90% of HR_{max} The tests were performed at 7- to 14-day intervals, in the following order: EXP 1 (normohydration, dehydration, and hyperhydration) and EXP 2 (normohydration).

7.2.1.4 Duration of exercise and exercise distances (Studies I-IV)

Exercise distances, shown in Table 4, were calculated on the basis of treadmill speed and duration of exercise.

Table 4. Duration of exercise and exercise distances in Studies I-IV.

Study I, II		Study IV (EXP 2)		Study IV (EXP 1)		Study III	
8 min	distance	**12 min**	distance	**40 min**	distance	**60 min**	distance
4-7 m/s	2 640 m	7 m/s	5 040 m	6 m/s	14 400 m	1.7-10 m/s	13 740 m
5-8 m/s	3 120 m	8 m/s	5 760 m	7 m/s	16 800 m		
6-9 m/s	3 600 m						
6 min							
5-7 m/s	2 160 m						

7.2.2 Experiments on circadian changes (Studies III, V)

In Study III, a 12-h time control experiment was carried out at the Equine Research Centre in Ypäjä, Finland, in five Finnhorses remaining in their boxes during the experiment.

In Study V, circadian variation in ANP, cortisol, and fluid balance was studied at the Faculty of Veterinary Medicine in Helsinki, Finland, in 10 goats remaining in metabolism cages during the experiment.

7.2.3 Samples

Blood samples were taken from the jugular vein through a plastic catheter. To avoid restraining any of the animals, the catheters were inserted about 1 h before each experiment. In Study IV (EXP 1), one sample before dehydration was drawn by venipuncture 24 h before the start of the exercise test.

Na- or K-EDTA was used as an anticoagulant in the sample for plasma hormone analysis and determination of PCV, and Ca-heparin in another sample for analysis of plasma concentrations of electrolytes, creatinine, total protein, and osmolality.

Aprotinin, a protease inhibitor, was added in the test tubes in Studies III and IV. Na-bisulfite was added to test tubes for determination of plasma catecholamines (Study II).

Urine was collected from goats via a Foley retention catheter inserted into the urinary bladder about 1 h before each experiment (Study V).

Table 5. Blood- and urine-sampling schedule.

1. Exercise studies			
	Before	**During exercise**	**After exercise**
Studies I, II	x	**8-min graded exercise** 2 min, 4 min, 6 min, 8 min	2 min, 5 min, 30 min 60 min
Study III	x	**60-min endurance exercise** 20 min (trot), 22 min (gallop), 52 min (trot), 60 min (canter)	60 min, 150 min, 270 min, 390 min
Study IV	x	**40-min steady-state exercise** 10 min, 20 min, 30 min, 40 min	5 min, 15 min, 30 min, 60 min, 120 min, 180 min
	x	**12-min steady-state exercise** 2 min, 4 min, 6 min, 8 min, 10 min, 12 min	5 min, 15 min, 30 min 60 min
2. Experiments on time-related changes			
	Before	**During**	
Study III	x	During 12 h 8 samples between 8:30 and 20:00	
Study V	x	During 24 h 3 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h, 24 h	

7.2.4 Other measurements

Heart rate was monitored during the exercise continuously with a pulse meter (Studies I, II, III), by obtaining a bipolar ECG (Study IV), or, once horses had left the treadmill, by phonoendoscopy (EXP 2 in Study IV). During exercise, data were recorded at the end of each interval or exercise phase.

Respiratory rate was monitored before and during exercise in EXP 2 (Study IV). Appearance of sweating was observed visually in Study II. Dehydration was estimated by weight loss (Hyyppä *et al.* 1996, Study IV).

7. 3 Analytical procedures

7.3.1 Blood and urine analysis

Plasma hormone concentrations were determined by RIA or HPLC. Prior to RIA, ANP, ADH, ACTH, and β-endorphin were extracted as described in Table 6. NT-ANP and cortisol were analysed in unextracted plasma.

Table 6. Hormonal analysis and methods.

Parameter	Method		Study
ANP	RIA	Peninsula Laboratories kit, Belmont, CA, USA; extraction by Geer *et al.* (1988)	I-V
NT-ANP	RIA	Vuolteenaho *et al.* (1985, 1992)	II, III
AVP	RIA	Vasopressin Rapid kit, Buhlman Laboratories AG, Allschwill, Switzerland; extraction by Geer *et al.* (1988)	II, V
ACTH	RIA	Vuolteenaho *et al.* (1981, 1992), Ekman *et al.* (1994)	II
b-endorphin	RIA	Vuolteenaho *et al.* (1981, 1992), Ekman *et al.* (1994)	II
catecholamines	HPLC	Kokkonen *et al.* (Study II)	II
cortisol	RIA	Cortisol (125I) Radioimmunoassay kit, Orion Diagnostica, Orion, Espoo, Finland;	II, V

The methods used for determination of PCV, total plasma protein, and plasma and urine osmolality, and the concentrations of Na^+, K^+, and creatinine are shown in Table 7.

Table 7. Plasma and urine analysis and methods.

Parameter	Method		Study
PCV	microhaematocrit	Adams Microhaematocrit centrifuge, Clay Adams Inc., NY, USA and ALC centrifugette 4203, Skafte & Claesson AB, Göteborg, Sweden	II, IV, V
total plasma proteins	light refraction	Atago Hand Refractometer, SPR-N, Japan and Refractometer, Cambridge Instruments, Buffalo, NY, USA	II, IV, V
osmolality (plasma, urine)	freezing point depression	Osmette A, Precision Systems Inc., Salsbury, MA, USA and 3W wide range osmometer, Advanced Osmometer, Inc., Roebling, Germany	II, IV, V
electrolytes (plasma, urine)	flame photometry	Corning Flame Photometry, Evans Electroselenium Ltd., Halstead, UK	II, V
creatinine (plasma, urine)	filter photometry	Kone Specific, Version 3.4, Konelab Oy, Espoo, Finland	V

In Study IV, the volume of excreted urine was measured. Urine osmolality and concentrations of Na^+, K^+, and creatinine were determined (Table 7). These variables were used for the calculations of urine electrolyte concentrations and renal clearances as described by Reece (1993).

7.3.2 Statistical analysis

Multivariate analysis of variance was used to test differences in responses to exercise between two horse breeds. The time-related data were analysed by ANOVA for repeated measures. Correlations were tested with regression analysis and by use of the Pearson correlation coefficient. A paired t-test was used to compare differences between pre-exercise values. Statistical significance was considered to be $P < 0.05$.

8. Results

8.1 Plasma ANP and NT-ANP levels (Studies I-V)

Plasma ANP concentrations in Standardbred and Finnhorse trotters (Study I and II), and in half-bred riding horses (Study III) were similar, as well as those in plasma NT-ANP concentrations in Finnhorses (Study II) and half-bred riding horses (Study III). The mean plasma ANP concentration in horses at rest was 5.7 ± 0.32 pmol/l (n=36), being 4.2 ± 0.27 pmol/l (n=6) in stallions, 6.5 ± 0.50 pmol/l (n=16) in geldings, and 5.5 ± 0.48 pmol/l (n=14) in mares. Plasma ANP was lower in stallions than in geldings ($P < 0.001$) and mares ($P < 0.05$). Plasma NT-ANP at rest averaged 232 ± 20.9 pmol/l (n=12). The plasma concentration in one stallion was 163 pmol/l, being low compared to the means of the geldings (263 ± 31.5 pmol/l, n=6) and mares (208 ± 27.1 pmol/l, n=5). The plasma NT-ANP/ANP ratio was 43 ± 7 (n=11). Plasma ANP concentrations for the goats (8.7 ± 0.86 pmol/l, n=10) were higher than those for horses ($P < 0.05$).

No significant difference in relation to age was observed in basal concentrations of plasma ANP in horses (n=32) or in goats (n=10), and in those of plasma NT-ANP in horses (n=12). Age of the horses and goats ranged from 3 to 13 years and from 2 to 8 years, respectively.

8.2 Responses to exercise

8.2.1 Plasma ANP and NT-ANP during and after exercise (Study I)

Plasma ANP concentration was measured in Standardbreds and Finnhorses before, during, and after SET on a treadmill. Heart rate was recorded before and during exercise.

Plasma ANP concentration was similar in both breeds during and after SET. During the 8-min graded exercise, plasma ANP increased linearly, by 6.7 ± 0.7 pmol/l, irrespective of physical condition, sex, and age, and peaked at 5 min post-exercise. At 30 min post-exercise, it was at the same level as at the end of exercise, and 30 min later, still significantly higher than before exercise. The resting heart rate was similar in both breeds and increased linearly, proportional to work intensity, reaching a mean level of 204 ± 3 bpm in both breeds.

8.2.2 Atrial peptides in relation to neuroendocrine responses and fluid balance (Study II)

Plasma concentrations of ANP, NT-ANP, ACTH, β-endorphin, AVP, catecholamines, and cortisol were determined before, during, and after SET. Plasma concentrations of total protein and electrolytes, plasma osmolality, and PCV were also measured. Heart rate was recorded before and during exercise.

The response of plasma ANP was accordant with that in Study I. NT-ANP increased significantly during exercise, but reached its maximum level at 30 min after exercise; 30 min later, it was still significantly above the level at the end of exercise. At rest, the NT-ANP/ANP ratio was 45 ± 9, but it fell significantly within 2 min after the start of exercise, returning by 1 h after exercise.

The increase in heart rate, up to 205 ± 5 bpm at the end of exercise, was similar to that in Study I. Plasma catecholamines and PCV increased immediately after the start of exercise, whereas increases in ACTH, β-endorphin, and AVP occurred more slowly. Cortisol increased significantly during exercise, but the elevation was only about 20 nmol/l. Plasma catecholamines and AVP decreased immediately after completion of exercise, whereas plasma ACTH, β-endorphin, and cortisol remained elevated longer. ANP correlated with noradrenaline at the onset of exercise. The increases in plasma ANP and AVP correlated negatively during exercise in four out of five horses. Changes in ANP did not correlate with those in ACTH, β-endorphin, or cortisol.

Sweating was mild and began at the late stage of exercise, being most prominent after exercise. PCV and plasma total protein, electrolytes, and osmolality increased

during exercise; within 5 min after exercise total protein and electrolytes returned to baseline, but PCV remained significantly increased. Assuming that exercise-induced change in PCV is affected by release of blood from the spleen, and that the decrease in plasma volume following SET is between 6% (Persson 1967) and 10% (McKeever *et al.* 1993a), it can be estimated on the basis of PCV that blood volume at the end of exercise may be expanded by 10 to 20% from the resting level (estimated blood volume at rest from 70 to 100 ml/kg, Swenson 1993).

8.2.3 Atrial peptides during and after repeated exercise under heat exposure (Study III)

Plasma ANP and NT-ANP were measured before, during, and after a 60-min endurance exercise test, which included two high-intensity phases. The changes in ANP were compared with those of aldosterone, AVP, and total plasma protein (Hyyppä *et al.* 1996) and of heart rate and plasma NT-ANP. Heart rate was recorded before, during, and at 30 min after CET.

No significant differences occurred in plasma ANP and NT-ANP levels among 5 exercise trials performed at an ambient temperature of +28 °C and a relative humidity of 58% in horses trained at a mean ambient temperature of + 7°C. ANP and NT-ANP increased significantly during exercise. In NT-ANP, the main increase occurred during the low-intensity phases (*Phases* C-X) including the trot and walk, whereas ANP remained unchanged during this period. ANP increased the most sharply during the high-intensity phases (*Phases* B and D) with the gallop and canter. Within 60 min after CET, ANP had fallen by 40% from the level at the end of CET, and NT-ANP by 11%. These reached the pre-exercise level 90 min later.

Exercise induced, in all five trials, a similar response in heart rate. During exercise, the heart rate varied with exercise intensity, and during the 30 min active recovery, heart rate decreased by 64%. ANP correlated neither with heart rate nor with plasma AVP during CET. The trends in mean ANP, NT-ANP, and aldosterone were quite similar during exercise, but were the opposite after 2.5 h of CET. Plasma protein increased by 11± 0.7 g/l during exercise, and 6.5 h later was still above the pre-exercise level. Body weight loss during CET among the five trials averaged 3.1% (Hyyppä *et al.* 1967).

8.2.4 Plasma ANP in relation to hydration status and exercise intensity (Study IV)
Plasma concentrations of ANP and total protein, plasma osmolality, and PCV were measured before, during, and after exercise, and respiratory rate before and during exercise. Heart rate was recorded before, during, and after exercise.

In EXP 1 and EXP 2, exercise increased ANP significantly. In EXP 1, plasma ANP showed a greater increase toward the end of exercise during hyperhydration than it did during dehydration or normohydration. The area under the plasma ANP concentration curve was significantly greater when the horses were hyperhydrated with 12 l of water than when they were dehydrated for 24 h before exercise (3% loss in body weight). In EXP 2, plasma ANP concentration remained increased for 30 minutes after exercise, whereas in EXP 1, plasma ANP concentration fell by approximately 30% during the same period. Body weight loss during exercise in EXP 1 was about 3%.

In EXP 1, heart rate before exercise was higher in the hyperhydrated horses than that in other horses, but in all trials, heart rate remained at a similar level during and after exercise. Within 5 min after exercise, heart rate decreased by about 30%. In EXP 2, heart rate decreased by 50% within 5 min, and within 30 min after exercise it had fallen by about 70%.

8.2.5 Effect of exercise type on plasma ANP and NT-ANP concentrations (Studies I-IV)
Plasma ANP and NT-ANP levels in horses at rest did not differ significantly between the Studies I-IV (Figs. 1, 2).

SET (Studies I, II) induced an equal increase in plasma ANP in Standardbreds and Finnhorses, irrespective of training status and of age ranging from 3 to 13 years. ANP increased during exercise by 7.0 $\pm$ 0.7 pmol/l. The rise was similar in the four Finnhorses, which performed a 6-min SET and started at a higher work intensity level than the other horses (Study I, Fig. 1). Following a 12-min steady-state exercise eliciting 90% of HR_{max} (heart rate about 200 bpm), the increase in plasma ANP in normohydrated horses was 9.9 $\pm$ 3.2 pmol/l (Study IV, EXP 2) and did not differ

significantly from the increases after SET (Fig. 1). During SET and EXP 2, the rates of change in plasma ANP were similar (1.7 ± 0.8 pmol/l/2min).

CET elevated ANP by 14.9 ± 2.0 pmol/l, significantly more than SET (Fig.1). A 40-min low-intensity exercise at constant treadmill speed (Study IV, EXP1, normohydration) raised plasma ANP by 10.9 ± 4.4 pmol/l, which did not differ statistically significantly from the increases during CET, SET, and EXP2. During EXP 1, the rate of increase in plasma ANP was 0.7 ± 0.8 pmol/l/2min.

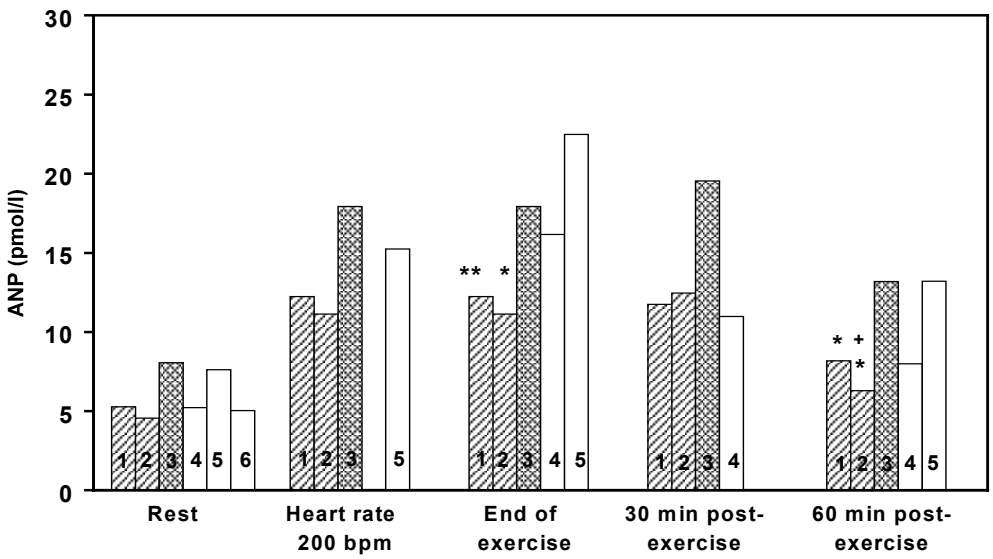

Figure 1. Changes in plasma ANP concentration in Studies I-IV: 1 = 8-min SET (n=20); 2 = 6-min SET (n=4); 3 = 12-min exercise at 90% of HR max (EXP 2; n=4); 4 = 40-min exercise at 65-70% of HR max (EXP 1; n=4); 5 = CET (n=6); 6 = mean 12-h plasma ANP level at rest (n=5). Values significantly different from bar 5 presented as * P < 0.05, ** P < 0.01, and from bar 3 as + P < 0.05.

At 30 min after SET and EXP2, plasma ANP was at the same level as at the end of exercise or slightly above this, but it fell by approximately 30% after EXP1 (Fig. 1). At 60 min after exercise, the relative decreases in ANP after endurance and short-term exercise tests were similar.

Plasma NT-ANP (Fig. 2) increased significantly more during CET than during SET. The post-exercise changes after CET and SET were significantly different; at 60 min after SET, NT-ANP rose by 166% from the level at the end of exercise, whereas at the same time, it fell by 11% after CET.

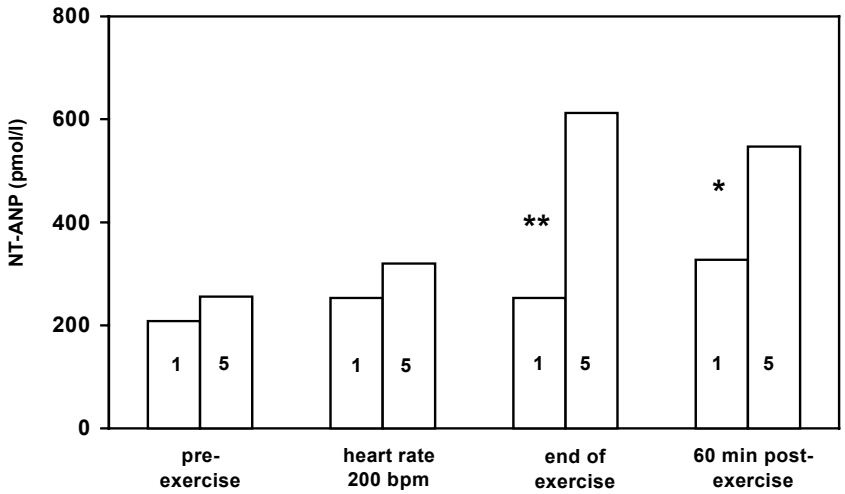

Figure 2. Effect of exercise type on plasma NT-ANP concentration. 1 = 8-min SET (n=5); 5 = CET (n=6). Values significantly different from bar 5 represented as * $P < 0.05$, ** $P < 0.01$.

8.2.6 Atrial peptides in relation to heart rate, treadmill speed, workload, and duration of exercise (Studies I-IV)

During SET, ANP correlated significantly with heart rate and treadmill speed, and NT-ANP with treadmill speed (Figure 3 a-b, Table 8). During CET, which included the exercise phases varying from walk to gallop, ANP and NT-ANP did not correlate with HR and treadmill speed (Study III). In Study IV, individual plasma ANP ranges were observed during steady-state exercise when the ANP concentrations were plotted against corresponding heart rate values (Study IV).

(a) (b)

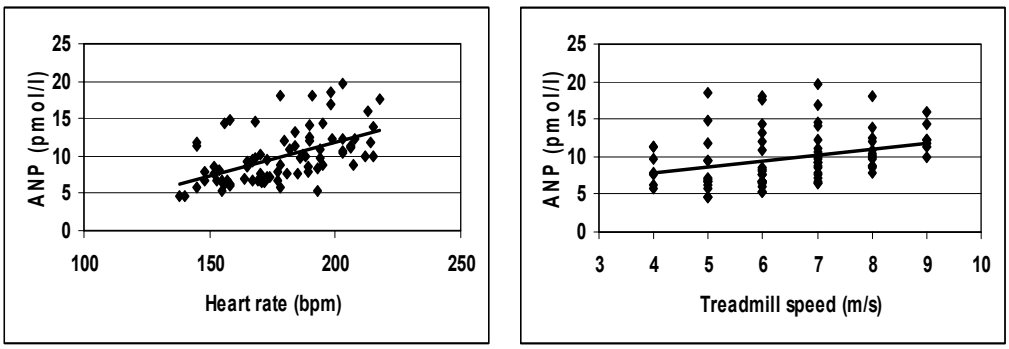

Figure 3. Scatter plot and regression line (a) ANP vs. heart rate, (b) ANP vs. treadmill speed in horses (n=20) during a 8-min submaximal graded exercise at four treadmill speeds from 4 to 9 m/s.

Table 8. Correlations of ANP and NT-ANP in horses with heart rate and treadmill speed during submaximal graded exercise.

	ANP vs. heart rate	ANP vs. treadmill speed	NT-ANP vs. heart rate	NT-ANP vs. treadmill speed
	Correlation coefficient	Correlation coefficient	Correlation coefficient	Correlation coefficient
all horses				
4-9 m/s, n=20	0,533 ***	0,319 **	nt	nt
Standardbreds				
6-9 m/s, n=7	0,623 ***	0,726 ***	nt	nt
Finnhorses				
5-8 m/s, n=6	0,457 *	0,519 **	nt	nt
Finnhorses				
4-7 m/s, n=7	0,461 **	0,428 *	nt	nt
Finnhorses[a]				
4-8 m/s, n=6			0,106	0,881 *

[a] the same Finnhorses as in ANP (2 of those in 5-8 m/s, 4 of those in 4-7 m/s)
* P < 0.05; ** P < 0.01; *** P < 0.001
nt = not determined

Table 9. Changes in ANP in relation to duration of exercise, exercise distance, relative workload, and heart rate in horses undergoing steady-state exercise (n=4, Study IV).

Time (min)	Distance (m)	% HR_{max}	Heart rate level (bpm)	Treadmill speed (m/s)	Change in ANP from 0 min (pmol/ml)	Maximal ANP (pmol/ml)
6	2340	90	201 ± 3	7 or 8	7.2 ± 1.9	
10	3900	90		7 or 8	7.5 ± 3.9	
12	4680	90	208 ± 3	7 or 8	9.9 ± 3.7	16 ± 6.0
20	7800	65-70	150 ± 5	6 or 7	2.4 ± 7.2	
30	11700	65-70	160 ± 7	6 or 7	5.9 ± 3.2	
40	15600	65-70	168 ± 3	6 or 7	8.1 ± 5.3	17 ± 4.0

8.2.7 Plasma ANP in relation to physical condition (Studies I-II)

Table 10 shows that, during exercise, physical condition may affect the heart rate and plasma ANP responses. A significant difference in plasma ANP concentration appeared when the concomitant difference in heart rate was 30 to 40 bpm, and the duration of exercise exceeded 2 min.

Table 10. Changes in heart rate and plasma ANP at treadmill speeds of 5 m/s and at 6 m/s in Standardbreds (n=7) and in well-trained (n=6) and less-well trained (n=7) Finnhorses.

	Duration of exercise (min)	HR (bpm)	ANP (pmol/l)
	Treadmill speed 5 m/s		
Finnhorses well-trained	2	152 ± 6	6.8 ± 1.1
less-trained	4	172 ± 6 §	10.3 ± 1.8
	Treadmill speed 6 m/s		
Standardbreds	2	156 ± 2	7.0 ± 0.3
Finnhorses well-trained	4	166 ± 6	8.2 ± 1.3
less-trained	6	195 ± 5 * * *	12.0 ± 1.8 * *

A significant difference between the Finnhorses § $P < 0.05$.
A significant difference between the less-trained Finnhorses and Standardbreds * * $P < 0.01$; * * * $P < 0.001$.

8.3 Cyclic changes in plasma ANP

8.3.1 Daily variation in ANP in horses (Study III)

In a time-control experiment, between 8:30 h and 20:00 h, ANP remained unchanged in 5 Finnhorses. At the individual level the daily variation was very small, and the horses retained their individual basal plasma levels throughout the experiment.

8.3.2 Circadian variation in ANP, cortisol and fluid balance in goats (Study V)

During the 24-h period, plasma ANP level ranged from 9.6 ± 0.8 pmol/l to 7.9 ± 0.7 pmol/l. Plasma concentrations of ANP, ADH, total protein, K, urine flow rate and osmolality, urine concentrations of Na, K, and creatinine, renal electrolyte excretion and clearances, and haematocrit showed no circadian variation. Circadian variation was observed in plasma osmolality, and the concentrations of Na and creatinine with achrophases around 16:00 h and nadirs between 01:00 h and 07:00 h. There was a small increase in plasma cortisol levels in 6 out of 9 goats at 04:00 h, which, however, was not statistically significant.

9. General discussion

9.1 Plasma ANP and NT-ANP levels in horses and goats

The molecular structure of ANP in mammalian species is highly conserved, and in these species, plasma concentrations, determined by existing methods from extracted plasma, are quite similar. The basal plasma levels in this study, ranging from 2 to 10 pmol/l, correspond well to the levels reported in healthy horses (McKeever *et al.* 1991a, b, 1992a, McKeever & Malinowski 1999) and goats (Olsson *et al.* 1989, Kokkonen 1992). Regardless of some physiological differences known to exist in Standardbreds and Finnhorses (Persson 1967, Pösö *et al.* 1983, 1987, Kline & Foreman 1991, Räsänen *et al.* 1993), the results of this study show that between horse breeds basal plasma ANP concentration does not differ.

Some hormones may be characterized by species-specific plasma levels, for example, cortisol with higher plasma concentrations in horses than in goats (Eriksson *et al.* 1994, Hydbring *et al.* 1996, Studies II and V). In ANP, as well, a slightly lower plasma level in horses than in goats may represent an interspecies difference. On

the other hand, the higher plasma ANP level of goats was observed only in female goats (Study IV). Thus, another factor that may explain this concentration difference is their gender. The study by Clark *et al.* (1990) shows in humans higher plasma ANP level in women than in men, and a similar trend, although not as marked, was observed in horses (Studies I-IV). However, a study in female goats (Olsson *et al.* 1989) has reported a lower ANP level than that of the present study, indicating that an analytical approach cannot be left out of consideration. Confirmation of the effect of gender on plasma ANP remains for further studies, since no other data on gender-related differences in ANP levels have been reported in horses or in goats.

The results by McKeever & Malinowski (1999) and those of the present study suggest that, in contrast to humans, in horses basal plasma ANP level does not increase with ageing (for reviews, see Freund *et al.* 1988, Cugini *et al.* 1992). The difference in age in the present study was not great, but McKeever and Malinowski compared horses which represented young (age about 5) and old (age about 22) individuals.

Plasma levels of bioactive compounds are determined by both release and clearance. In all species studied, ANP_{99-126}, the carboxy-terminus of $proANP_{1-126}$, is secreted into the circulation in equimolar amounts with NT-ANP_{1-98}, the amino-terminus of $proANP_{1-126}$ (Vuolteenaho 1985, Itoh *et al.* 1988, Ruskoaho 1992), but apparently due to their different elimination, the molar ratio of NT-ANP/ANP in peripheral plasma is higher than expected on the basis of knowledge on their release (Arjamaa *et al.* 1996), an observation made also in this study. The amino acid sequence of equine NT-ANP_{1-98} is highly homologous with that of other species (Richter *et al*. 1998, Vlasuk *et al.* 1986), and its basal plasma levels, although measured only in the present study, seem to be similar to those in humans (Sundsfjord *et al.* 1988, Buckley *et al.* 1989, Baker *et al.* 1990, Vuolteenaho *et al.* 1992, Arjamaa *et al.* 1996). Furthermore, comparable to ANP, levels of plasma NT-ANP between horse breeds were similar (Studies II, III).

Excitement, anxiety, and fear appear to have an influence on cardiovascular responses and haematological values, and on plasma hormone concentrations (Stewart *et al.* 1977, Rose & Hodgson 1982, Revington 1983, Erickson 1993). The

animals in the present study were easily trained for the experimental conditions and seemed to be well accustomed to them. However, the increases in plasma cortisol and AVP concentrations at the end of Study V may indicate restlessness at this time-point. These changes were not accompanied by any increase in ANP in goats, possibly because the goats were physically restrained. Conversely, in one horse the elevated plasma ANP value at the end of the time-control experiment (Study IV) may have resulted from increased physical activity and apprehension related to the last sampling in the box where the horse, between the samplings, was free to move. Otherwise, no stress of any kind was expected to affect results significantly.

9.2 Changes in plasma ANP and NT-ANP in horses during exercise

Cardiovascular adaptation to exercise is characterized by an increase in cardiac output as a result of the combined effect of sympathetic and parasympathetic responses, and the muscle pump and respiratory pump (Stephenson 1997). Sympathetic activation and catecholamines (Study II) affect cardiac function by increasing heart rate (Studies I-IV) and force of contraction, as well as by shortening the duration of systole, which preserves the diastolic filling time (Stephenson 1997). They also cause contraction of the splenic capsule and vasoconstriction of the splenic vascular bed, resulting in release of stored blood from the spleen into the circulation (Davies & Withrington 1973, Erickson 1993) and, thereby, in an marked increase in PCV (Study II, IV). Increased venous return along with an increase in ventricular preload assists an increase in end-diastolic volume and stroke volume by the Starling mechanism (Erickson 1993, Stephenson 1997). Heart-rate levels obtained in Studies I to IV have been shown in horses to be associated with significant haemodynamic changes, including an increase in right atrial pressure (Hopper *et al.* 1991, McKeever *et al.* 1993b, Marlin *et al.* 1996). Considering the mechanism related to cardiovascular acceleration during exercise, and that atrial muscle stretch is regarded as a central regulator of ANP release (Ruskoaho 1992, de Bold *et al.* 1996), and is shown to stimulate ANP release from cardiocytes both *in vitro* and *in vivo* (Dietz *et al.* 1984, Lang *et al.* 1985), it is not surprising that exercise raises plasma ANP concentration in horses (McKeever, 1991 a, b, 1993b, Studies I-IV), as well as in humans (Fyhrquist *et al.* 1987, Espiner *et al.* 1986, Somers *et al.* 1986, Tanaka *et al.* 1986, Saito *et al.* 1987, Baker *et al.* 1991, Vuolteenaho *et al.* 1992) and in other species (Miller *et al.* 1990, Mäntymaa *et al.* 1994). Many factors

such as intensity and duration of exercise, training, ageing, and various disease states have been reported to modulate responses of atrial peptides and other hormones to exercise (Fyhrquist *et al.* 1987, Freund *et al.* 1988, Malinowski & McKeever 1999). Some of these factors will be discussed in this study.

The results of this study support the view that the principle of the mechanism controlling ANP release is similar in horses and in other species. However, physiological pecularities, which, in the horse, are related to excellent aerobic performance capacity (Erickson 1993), may be of importance in modulating responses of atrial peptides. For example, exercise-induced blood volume expansion, which is significant in horses (Persson 1967), and which in them may play an important role in releasing atrial peptides, remains minor in humans due to the lower capacity of the human spleen to release blood to compensate for the exercise-induced decrease in plasma volume (Green *et al.* 1984, Laub *et al.* 1993). Furthermore, it is well known that the horse, due to the large volume of its intestine, has a great capacity for fluid shift from the gastrointestinal tract (Argenzio 1993), a phenomenon which in theory may have effect on responses of atrial peptides.

Plasma concentration of a hormone is determined by both release and clearance. Clearance of atrial peptides involves binding to clearance receptors found in various organs (Hollister *et al.* 1989, Gerbes & Vollmar 1990) and involves enzymatic degradation by neutral endopeptidase or some other enzymes (Gerbes & Vollmar 1990). The potency of degradation is dependent on the tissue and is higher in kidney than in liver or lung (Tang *et al.* 1984). During exercise, visceral blood flow decreases, whereas blood flow in working muscles increases, as shown by Parks & Manohar (1983) in ponies and Manohar *et al.* (1995) in horses. The blood flow in different organs determines their contribution to the total body clearance of atrial peptides (Gerbes & Vollmar 1990). Thus, increased circulation, by enhancing exposure to clearance receptors, may accelerate ANP clearance. No pharmacokinetic information on atrial peptides in horses has been published, but the results in Study II led us to assume that the half-life of ANP during exercise is short, being about 30 times less than that of NT-ANP. For NT-ANP, the kidney is the major site of degradation (Katsube *et al.* 1986, Itoh *et al.* 1988, Sundsfjord *et al.* 1988), but

it has not yet been established whether redistribution of blood, or impairment of glomerular filtration rate (Gleadhill *et al.* 2000) alter its elimination in horses.

9.3 Changes in plasma ANP and NT-ANP in horses after exercise

The temporal patterns of plasma ANP and NT-ANP suggest that their release continues to be increased after completion of exercise (Studies I-IV). This differs from the situation in humans (Fyhrquist *et al.* 1987, Espiner *et al.* 1986, Somers *et al.* 1986, Tanaka *et al.* 1986, Saito *et al.* 1987, Baker *et al.* 1991, Vuolteenaho *et al.* 1992). However, the different experimental approaches and the marked difference in exercise capacity between horses and humans make these comparisons problematic.

The rapid decrease in plasma catecholamines and in heart rate after completion of exercise (Study II), shows that immediately after completion of exercise, sympathetic activity of the heart is diminished. However, unlike in humans (Somers *et al.* 1986), the heart rate in horses may be slightly elevated more than 30 min after exercise (Sexton & Erickson 1990, Geor *et al*. 1995, Study III, IV), but at such low rates it is unlikely *per se* to be contributory toward in stimulating ANP secretion. Another factor involved may be hypervolemia, which returns slowly due to prolonged post-exercise refilling of the spleen (Persson 1967). Together with fluid shifts into the intravascular compartment (Nyman 2001), it may keep venous return high for some time after exercise. Moreover, the inspiratory pump remains activated in horses after completion of exercise (Manohar *et al.* 1994, Study IV) and may contribute to venous return. Hormonal factors, however, seem not to be responsible for the post-exercise changes in atrial peptides (Study II). Since these factors only partially explain the long-lasting increase in atrial peptides, the contribution of other factors, vasoactive or biochemical, warrants consideration.

As stated, plasma half-lives of ANP and NT-ANP are supposed to differ, which in addition to continued release of these peptides may explain their temporal pattern also after exercise (Study II). Since these are the first results that describe post-exercise changes in atrial peptides in horses, and data on pharmacokinetics of atrial peptides, as well as on post-exercise haemodynamics and renal function in horses are limited, the importance of this suggestion remains to be established.

The more rapid return of plasma NT-ANP after CET than SET (Study II, III) suggests that dehydration induced by exercise may alter the recovery of atrial peptides. This is supported by earlier results in horses which have shown that fluid balance has an effect on the exercise-induced plasma volume response (Hopper *et al.* 1991, Nyman 2001), which, in turn, has been shown to alter the plasma atrial peptide response (Hichcliff & McKeever 1998, Study IV). Experimental manipulations that induce changes in blood volume and pressure have been found to regulate the density of clearance receptors and plasma ANP level (Ruskoaho 1992). For example, in rats a short water deprivation was reported to increase ANP_C receptor density on glomerular membranes and to decrease plasma ANP levels (Kollenda *et al.* 1990). It is not known whether or how changes in clearance receptor density contribute to alterations in plasma levels of atrial peptides in horses.

9.4 On factors associated with changes in plasma atrial peptides

9.4.1 Heart rate

Experimental evidence exists that chronotropic stimulation increases ANP release (for references, see Ruskoaho 1992). Moreover, results in exercising human subjects (Somers *et al.* 1986, Tanaka *et al.* 1986, Fyhrquist *et al.* 1987, Saito *et al.* 1987) and horses (McKeever *et al.* 1991b, Studies I, II) show that exercise, which accelerates heart rate, increases plasma ANP concentration proportionally to the change in heart rate. However, the present results suggest that the increase in heart rate may only partly explain the variability in plasma ANP values during exercise (Studies I, IV); this is in agreement with the theory that heart rate *per se* is not the main stimulus for the ANP secretion (de Bold *et al.* 1996). *In vivo*, for example during exercise, tachycardia is accompanied by increases in atrial pressure and pulmonary wedge pressure, which also may explain the elevation in ANP (Walsh *et al.* 1987, Walsh *et al*. 1988, Hinchcliff *et al.* 1990, Hopper *et al.* 1991). However, the observation in splenectomised horses that heart rate and plasma ANP increase in response to exercise without a simultaneous increase in right atrial pressure (McKeever *et al.* 1993b) indicates that increased preload alone cannot explain the exercise-induced changes in ANP. However, these results agree well with the concept that atrial stretch and atrial wall tension/stress explain the tachycardia-induced stimulation of ANP (Ruskoaho 1992). The lack of correlation between ANP and heart rate during

CET (Study III), which is known to increase right atrial pressure (Marlin *et al.* 1996), may possibly be explained by bias caused by the fate of ANP after the high-intensity phase of exercise, comparable to that after SET.

Moreover, a decrease in heart rate after completion of exercise (Sexton & Erickson 1990, Sheerman & Morris 1990, Lovell & Rose 1995, Study IV), and variable changes in plasma ANP (Studies I-IV) support the view that during recovery primarily other factors than heart rate account for the post-exercise changes in plasma ANP concentration.

9.4.2 Blood volume expansion

In horses, exercise causes hypervolemia due to release of splenic blood into the circulation (Persson 1967, Persson *et al.* 1973). The emptying of the spleen is related to workload and occurs rapidly at the start of exertion (McKeever *et al.* 1993a), suggested also by the sharp increase in PCV at the early stage of exercise (Studies II). On the other hand, exercise reduces plasma volume (Persson 1967, Masri *et al.* 1990, McKeever *et al.* 1993a, Lindinger *et al.* 1995, Studies II, IV), as indicated by the increases in plasma concentrations of total protein and Na as well as in plasma osmolality. If the decrease in plasma volume is similar to that reported for a comparable type of exercise (Persson 1967, McKeever *et al.* 1993a), it can be estimated that the increase in blood volume during short submaximal graded exercise was 10 to 20% of total blood volume. This is an amount sufficient to increase ANP release, as shown by numerous studies *in vitro* and *in vivo* (for references, see Ruskoaho 1992). In keeping with this, the studies by McKeever *et al.* (1991c, 1993b) have shown in horses that the splenic release of blood increases right atrial pressure and plasma ANP concentration. The relationship between ANP and right atrial pressure in horses has been subsequently confirmed (Hinchcliff & McKeever 1998).

Hypervolemia combined with respiratory and cardiovascular adjustments may also serve as an explanation for increased atrial peptide levels for some time after exercise. Re-filling of the spleen is known to be slower than its emptying, requiring at least 20 minutes in horses (Persson 1967), which agrees with the observations in Study II. Because the post-exercise decrease is faster in total protein than in PCV

(Sheerman & Morris 1990, Study II, IV) it can be speculated that plasma volume is restored after exercise and that refilling of the spleen occurs even more slowly than expected on the basis of PCV values only. The absence of any comparable exercise-induced volume expansion in humans leads to speculation that this might partly explain the difference in recovery of plasma atrial peptides between horses and humans.

9.4.3 Hormones

Interactions of endocrine factors with changes in atrial peptides were examined in Studies II and III. Noradrenaline was found to correlate with ANP at the the start of exercise, and it may be involved in ANP release during exercise (Study II), indirectly through the splenic function or directly, as suggested by experiments *in vitro* (Schiebinger *et al.* 1987). However, the rapid return of catecholamine concentrations after exercise suggest that they are not involved in ANP release during recovery. During and after exercise, plasma cortisol concentration increased, reflecting the changes in plasma ACTH (Study II). Glucocorticoids have been shown to increase circulating ANP levels and to increase synthesis of both ANP mRNA and ANP prohormone (Gardner *et al.* 1986, Argentin *et al.* 1991). However, because of the short time-interval of Study II, their effect on plasma atrial peptide level may have been small. Pressor hormones, such as AVP, increase plasma ANP, either by increasing atrial distention or by enhancing stretch-mediated ANP release (Ruskoaho 1989b, Ruskoaho 1992), and may have contributed to ANP release Studies II, III). However, no correlation was observed between ANP and AVP during CET (Study III), and a negative correlation was observed during SET (Study II), possibly due to volume load, known to raise plasma ANP concentration and to suppress AVP release (Schrier *et al.* 1979, Ruskoaho 1992). Because the endocrine responses do not fully explain the increased release of atrial peptides during and after exercise, it is possible that enhanced release of natriuretic peptides following acute stimulation**,** which was studied here, is based primarily on mechanical activity of the heart and not on changes in the hormonal environment; the latter, however, may become important during chronic stimulation (de Bold *et al.* 1996).

9.4.4 Heat exposure

Repeated exercise bouts at +28 °C ambient temperature and 58% relative humidity during 8 weeks did not alter plasma ANP or NT-ANP at rest nor their responses to exercise in horses not acclimated to heat before the experiment (Study III). Because the exercise sessions were performed at 2-week intervals, and the horses were stabled and trained in a cool environment, it seems possible that the benefits of short-term heat acclimation were lost between the experiments, as is reported to occur in human beings after 7 days without heat exposure (Armstrong *et al.* 1991). Plasma volume expansion, which occurred during the 8-week period (Hyyppä *et al.* 1996), complicates the estimation of absolute changes in atrial peptides. On the other hand, the work of Lindinger *et al.* (1995) suggests that heat and humidity (+30-34°C/80-85% relative humidity) *per se* do not alter plasma volume and ion responses to an acute exercise bout, as compared to cool and dry conditions (+22°C/45-55% relative humidity).

9.4.5 Fluid balance

The data by Hopper *et al.* (1991), Hinchcliff & McKeever (1998), and from Study IV indicate that changes in atrial filling pressure induced by contraction or expansion of plasma volume alter right atrial pressure and plasma ANP concentration. Furosemide administration (Hinchcliff & McKeever 1998) and water deprivation (EXP I, Study IV), which resulted in a decrease in plasma volume as indicated by a decrease in total plasma protein, attenuated plasma ANP response to exercise. This is consistent with several other investigations without exercise which have shown reduction in plasma ANP following fluid loss and decrease in plasma volume (for reviews, see Ruskoaho 1992). Conversely, an increase in plasma volume, which occurs during hyperhydration (Study IV), increases the response of ANP to exercise, apparently through increased right atrial pressure (Hopper *et al.* 1991, Hinchcliff & McKeever 1998).

Sweating affects fluid balance by causing dehydration and decrease in plasma volume (Carlsson 1983, McCutcheon *et al.* 1995, 1996). During CET (Study III), loss in body weight averaged 3.1%, at the same magnitude as reported earlier in a comparable exercise test in horses exercising in hot, dry (+30°C/40% relative humidity) and in hot, humid (+30°C/85% relative humidity) ambient conditions (Marlin

et al. 1996). This figure was slightly, and nonsignificantly greater than the decreases (2.4%) after CET in cool and dry (+20°C/40% relative humidity) conditions (Marlin *et al.* 1996), or than (2.8%) after steady-state endurance exercise (EXP 1 in Study IV). The nearly similar effect on fluid balance may partially explain the similarity in ANP responses during endurance exercise in CET and in EXP1.

The results of Study IV suggest that water balance may modulate plasma atrial peptide concentrations after exercise, possibly by altering atrial filling pressure. This is supported by results in Studies II and III, in which the effect of altered water balance was best seen as a more rapid decrease in atrial peptides after CET, which caused a marked dehydration, than after SET, which failed to make any considerable change in water balance. In this respect, plasma NT-ANP appeared to serve as a better indicator.

9.4.6 Exercise intensity

Increase in plasma ANP concentration has been related to exercise intensity in horses (McKeever *et al.* 1991b, Studies I, II, IV) and in humans (for references, see Freund *et al.* 1988). Studies I and II show a linear relationship betweeen ANP and increasing work intensity, the trend observed also in plasma NT-ANP (Study II). However, reduction in exercise intensity during exercise did reduce plasma atrial peptide concentrations (Study III), based on the well-known fact that the plasma level of a hormone is determined by both release and clearance. In addition to effects during exercise, exercise intensity was shown to affect the post-exercise return of atrial peptides to basal plasma levels (Studies I-IV).

9.4.7 Training

Plasma ANP concentration remains unchanged after training periods lasting from 4 to 55 weeks in dogs and rats (Mäntymaa 1994) and in humans (Freund *et al.* 1987, Vollmer-Larsen *et al.* 1989). Available data suggest also that training does not alter basal levels of ANP, either in endurance-trained subjects or in untrained controls (Freund *et al.* 1988), or in endurance athletes after a 4-week training period (Vollmer-Larsen *et al.* 1989), or in trained horses after a training period of 8 weeks (Study III), or in comparison with ANP levels in trained horses with differing training levels (Studies I, II). Plasma volume expansion following training (McKeever *et al.* 1987,

Hyyppä *et al.* 1996) may complicate estimation of absolute changes in plasma hormone levels and mask any alteration in their secretion and/or elimination.

Degree of training has an influence on performance capacity and fitness (Persson 1983). It has been shown that training does not change the level of maximal heart rate but reduces the steepness of slope of the regression line of submaximal heart rate versus treadmill speed which in well-trained individuals results in a higher work intensity to reach a heart rate of 200 bpm (Rose & Evans 1987), an observation made also in Studies I and II. The findings of plasma atrial peptide levels related to heart rate and work intensity (Studies I, II) and their different correlations between Standardbreds and Finnhorses as well as between the groups of well- and less well-trained Finnhorses support the view that physical condition has an effect on plasma ANP and NT-ANP responses in horses. To cause a significant difference in plasma ANP, the difference in increase in heart rate must exceed 30 bpm and the duration of exercise 2 minutes. As shown in Study I, the effect of physical condition on test results can be avoided by standardizing the test on the basis of the heart rate response and duration of exercise.

9.4.8 Circadian changes

Circadian rhythm is characteristic of many physiological variables. This should be considered, especially when assessing test results which have a range near the level of the circadian changes. In horses, secretion of catecholamines, cortisol, and possibly β-endorpin exhibits circadian rhythmicity (James *et al.* 1970, Hamra *et al.* 1993, Irvine & Alexander 1994, Kurosawa *et al.* 1997, Mehl *et al.* 1999). In the present study, exercise-induced responses were much higher than the expected circadian changes, and thus any influence of circadian variation on responses was obviously minor. Instead, individual plasma hormone levels characteristic of many hormones were observed also in the the present study.

Plasma ANP concentration was unchanged in resting horses as well as in goats standing or lying down for 24 h. These observations support the view that in ANP no circadian variation with a classic day-night pattern or any other cycle exists. However, data on circadian variation in ANP is conflicting, since some studies have reported circadian rhythm in plasma ANP in humans (Donckier *et al.* 1986, Winters *et al.* 1988,

Bell *et al.* 1990, Vesely *et al.* 1990, Cugini *et al.* 1992, Sothern *et al*. 1996) and rats (Kanervo *et al.* 1991, Oliveira *et al.* 1993), whereas others have found no circadian fluctuations (Richards *et al.* 1987, Follenius *et al.* 1992, Chiang *et al.* 1994, Kool *et al.* 1994). Wolf *et al.* (1990) reported in calves two daily increases in ANP, but these were possibly due to feeding or to alteration in plasma volume, and not due to actual circadian rhythm. On the other hand, the results of Wolf *et al.* (1990) point out the involvement of varying factors causing circadian fluctuation in ANP. Consequently, it can be speculated that also reasons other than circadian variation may explain circadian changes in humans and rats. For example, postural changes are known to affect plasma ANP level (Gillies *et al.* 1987, for other references, see Ruskoaho 1992). Since human being and rats consistently use both vertical and horizontal postures, unlike quadrupeds, part of the divergency in the results may be posture-related. In keeping with this, Richards *et al.* (1987) and Follenius *et al.* (1992) demonstrated under controlled experimental conditions the absence of circadian variation in humans who remained in a recumbent position for 24 h. It is noteworthy that the horse and goat, used in the present study, are posturally more inactive than the human and rat and during the experiments were physically inactive (Studies IV, V), like the humans in the two studies mentioned above. Circadian changes in NT-ANP are unknown, but considering the secretion mechanism of atrial peptides, it is probable that the changes are consistent with those for ANP.

10. Conclusions

Atrial natriuretic peptides, COOH-terminal ANP_{99-126} (ANP), and the NH_2-terminal NT-ANP_{1-98} (NT-ANP), are continuously secreted by the heart. On the basis of the present study in the horse and goat, the following characteristics were shown:

The basal plasma ANP concentration levels in the horse and in the goat were low, ranging from 1 to 10 pmol/l, and were well comparable to those in healthy individuals of mammalian species. The basal plasma NT-ANP concentration ranged in horses from 200 to 300 pmol/l, being approximately 45-fold more than that of ANP. These results provide no evidence for any inter-breed differences in basal plasma concentrations of atrial peptides, or for any alteration following physical training. In mares and geldings, plasma concentrations seemed to be higher than in stallions. No circadian variation with a classic day-night pattern or any other cycle was observed in plasma ANP in goats, and during daytime, the plasma ANP concentration was remarkably constant also in resting horses. The results suggest that in ANP no circadian rhyhm exists.

Exercise increased the plasma concentrations of ANP and NT-ANP, but at different temporal rates, most probably due to differences in their plasma half-lives. The increase in ANP was linear with increasing heart rate and treadmill speed (work intensity). Exercise increased venous PCV, apparently due to mobilization of the splenic blood cell reservoir. Consequently, blood volume expansion with muscle movements and inthrathoracic pressure could be expected partially to explain the increase in plasma atrial peptides. Plasma concentration of pressor hormones increased during exercise, but except for the correlation between noradrenaline and ANP, their interplay with atrial peptides could not be demonstrated.

In horses, exercise induced a long-lasting post-exercise increase in plasma ANP and NT-ANP, which differs from the observations in human subjects. The increase in NT-ANP after exercise indicates that the release of atrial peptides is stimulated after completion of exercise. The role of vasoactive hormones seems to have been minor. Instead, the elevation in central blood volume appeared to be a possible explanation for the increase in atrial peptides during early recovery from exercise. Because the

half-life of ANP seemed to be short and its return slow, it is possible that other factors were involved, but their nature could not be determined in these studies.

Repeated exercise bouts at 2-week intervals at 28°C and 58% relative humidity did not alter plasma ANP and NT-ANP resposes in horses stabled and trained in a cool (7-16°C) ambient temperature.

Difference in water balance, as shown by dehydration, normohydration, and hyperhydration, was shown to modulate plasma ANP concentration during exercise and recovery. The results showed also that return of atrial peptides was slower after short-term submaximal exercise than after endurance exercise, which caused a marked dehydration.

Exercise intensity was shown to modulate post-exercise changes in plasma atrial peptides.

In addition, the results provided evidence that differences in performance capacity and fitness within or between the breeds may be followed by varying responses in plasma atrial peptide concentration. Standardization of exercise tests according to heart-rate response can abolish these differences.

Taken together, the results of these studies support the theory that an increase in plasma ANP is related to factors that increase atrial stretch. This is consistent with the prevailing view and suggests that ANP is involved in regulation of cardiovascular control and fluid balance during and after exercise in horses.

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