The Role of Arginine-Vasopressin in Blood Pressure Control: Successful Breeding of Hypertensive Rats with a Single Genetic Defect in Arginine Vasopressin (AVP) Synthesis. W. Rascher*, U. Ganten, D. Ganten, R.E. Lang, Th. Unger, R. Taugner**, R. Rettig, R. Dietz. German Institute for High Blood Pressure Research and Department of Pharmacology, * Department of Pediatrics, ** Department of Physiology, University of Heidelberg, Federal Republic of Germany.

Introduction

The classical concept of the hormonal action of arginine vasopressin (AVP) is based on its secretion from the posterior pituitary gland into the blood and its antidiuretic action on the kidney as the main target organ. The pathophysiological role of neuronal pathways originating from the paraventricular and supra-chiasmatic nucleus and projecting to extrahypothalamic sites is less clear. This paper is dealing with the cardiovascular effects of neuronal and hormonal AVP. Oxytocin (OXT) will be briefly included in the discussion.

Hormonal plasma AVP appears to have marked cardiovascular activity besides its action on the kidney. Central AVP seems to counteract, at least in part, the actions of circulating AVP (Liard et al., 1981; for review see Rettig et al., 1982). The pressor effects of AVP on one hand and the pressor inhibiting action on the other suggest that this peptide can activate blood pressure increasing but also blood pressure lowering pathways. This may find an explanation by the fact that AVP has been shown to be stimulated by isoosmotic hypovolaemia as well as by normoor hypervolaemic hyperosmolality. In the first case (e.g. following haemorrhage) an antidiuretic and pressor action of AVP would be meaningful to regain volume homeostasis and to improve the haemodynamic situation. In the case of stimulation of AVP by hyperosmolality a depressor action together with renal water reabsorption might be the more adequate response, since there is no volume deficit and blood pressure may be normal or already increased.

In the first part of this paper we are discussing the distribution and cardiovascular actions of AVP; in the second part we will report on the successful breeding of a new line of spontaneously hypertensive rats with a complete lack of AVP synthesis. The data allow conclusions concerning the role of AVP in blood pressure control.

Distribution and cardiovascular actions of Vasopressin

AVP and oxytocin (OXT) are peptides synthesized in the hypothalamus and released via the neurohypophysis into the circulation. AVP has long been known for its potent antidiuretic and vasopressor properties and OXT for its role in birth and lactation.

The presence of separate AVP- and OXT-containing neurons in the brain has been demonstrated by immunohistochemical techniques in various extrahypothalamic brain areas, including the forebrain, brainstem and spinal cord. The supraoptic nucleus mainly projects into the posterior hypophysis, whereas the AVP containing fibres originating from the supra-chiasmatic nucleus almost exclusively travel into extrahypothalamic brain regions, e.g. septum, thalamus and lateral habenula (Sofroniew and Weindl, 1978). The paraventricular nucleus subserves both pituitary and extrahypothalamic neurons, e.g. hippocampus, amygdala, dorsal motor nucleus of the vagus, nucleus tractus solitarius (NTS), nucleus ambiguus and substantia gelatinosa of the spinal cord (Buijs, 1978; Nilaver et al., 1980). These immunohistochemical findings were corroborated by quantitative measurements which revealed remarkably high concentrations of AVP and OXT in the medulla oblongata and in other extrahypothalamic brain areas (Dogterom et al., 1978; Glick and Brownstein, 1980), and the radioimmunologically detected activity has been identified by means of high pressure liquid chromatography in combination with radioimmunoassays (Lang et al., 1982; Lang et al., 1983). Little is known about the functional role of extrahypothalamic AVP- or OXT-containing neurons. Central AVP has been shown, however, to be related to behavioral effects (De Kloet and De Wied, 1980), particularly to the facilitation of memory consolidation (Kovacs et al., 1979).

The intracellular localization of AVP and OXT in and around important cardiovascular control centers suggests a role of these peptides in the central regulation of arterial blood pressure, although the experimental data are still contradictory. Evidence for a central cardiovascular action of plasma AVP comes from experiments comparing infusions of the peptide into the inferior vena cava and into the vertebral artery of conscious dogs (Liard et al., 1981). In this study heart rate decreased to a greater extent after intravertebral as compared to intravenous drug infusion, despite the fact that increases in arterial pressure were less after intravertebral administration of AVP. These findings could be interpreted to the effect that AVP interferes centrally with the baroreceptor reflex to decrease cardiac output. This central depressor effect counteracts the peripheral vasoconstrictor activity of AVP.

Early experiments employing intracerebroventricular (i.c.v.) injections of crude posterior pituitary extracts in cats and dogs did not result in clearcut effects. If anything, a tendency towards blood pressure decreases and bradycardia was recognized (Nashold et al., 1962; Varma et al., 1969). However, in a recent study Pittman et al., (1982) observed a rise in blood pressure of short latency following i.c.v. injections of AVP (25 μ mol to 5 μ mol) in anaesthetized Sprague Dawley rats and in rats homozygous for hypothalamic diabetes insipidus (DI) which completely lack endogenous AVP. On the other hand, injection of AVP or OXT into the lateral brain ventricle of rats attenuated the pressor response evoked by electrical stimulation of the mesencephalic reticular formation (Bohus et al., 1982). Microinjections of AVP into the hippocampal dentate gyri imitated the effect as seen after i.c.v. administration of the drug, indicating that this brain region may play a role in the mediation of depressor effects elicited by central AVP. When injected into the fourth ventricle, OXT or fragments of it proved to be even more potent, whereas AVP administered by the same route had no effect. This points to different target sites of the two peptides for the modulation of cardiovascular regulation.

Apart from the hippocampus, brain stem nuclei have been speculated to be possible target sites for centrally acting AVP. Among these the NTS has attracted particular attention, because it has been shown to receive AVP-containing fibres from the suprachiasmatic and paraventricular nucleus (Sofroniew and Weindl, 1978; Buijs, 1978; Nilaver et al., 1980), and because it represents the first synapse of the afferent limb of the baroreceptor reflex arch.

A tonic inhibitory influence of AVP-synthesizing nuclei in the hypothalamus on the baroreceptor reflex was suggested by Ciriello and Calaresu (1980) on the basis of their observation that electrical stimulation of the paraventricular and supraoptic nucleus in the cat inhibited the reflex bradycardia following carotid sinus nerve stimulation.

Microinjections of AVP into the NTS of anesthetized rats have revealed dose-dependent increases in blood pressure (Matsuguchi et al., 1980), whereas a marked depressor effect was found after microinjection of the AVP antagonist d(CH₂)₅AVP. Thus, while AVP appears to inhibit pressor effects in the hippocampus, it has blood pressure increasing effects in the brain stem and in periventricular regions.

The role of brain AVP in hypertensive mechanisms was studied by measurements of AVP in the brain of stroke-prone spontaneously hypertensive rats (SHRSP). AVP content in the brain stem and in the hypothalamus was found to be lower than in normotensive controls (Möhring et al., 1980; Lang et al., 1981a; Rascher et al., 1982). By crossing SHRSP and rats homozygous for diabetes insipidus, we have recently been able to breed a new line of genetically hypertensive rats which is completely deficient in AVP. These experiments will be reported in some more detail.

Breeding of a new line of hypertensive rats with hereditary hypothalamic diabetes insipidus

The qualitative and quantitative study of the importance of hormones or neurotransmitters in pathophysiological processes is severely hampered by the diffi-

Fig. 1
Genealogical outline of selective breeding of SHRSP with rats homozygous for hereditary hypothalamic diabetes insipidus with stepwise accumulation of SHR genes and a reappearance of DI animals from the di/+ x di/+ intercross. The following symbols and abbreviations have been used: Brattleboro rats, homozygous for hypothalamic diabetes insipidus (DI), genetic code: di/di; Brattleboro rats, heterozygous for hypothalamic diabetes insipidus (HZ), genetic code: di/+; Long Evans control rats (LE), genetic code: +/+. Values on top of symbols indicate mean arterial blood pressure (MAP) of corresponding generation, parental (P) and filial (F) generations are indicated on the left. Heavy outlines indicate rats with di/di gene.

culties to study their actions without interfering at the same time with an virtually unlimited number of other regulatory systems. The breeding of animals with one selective defect of hormonal gene expression is one promising way to circumvent these experimental problems. Rats with a hereditary defect in arginine vasopressin (AVP) synthesis have thus proven to be a useful animal model to study volume homeostasis.

It appeared therefore that the introduction of the DI-gene into spontaneously hypertensive rats might help to solve the controversies concerning the role of AVP in experimental hypertension (Johnston et al., 1981).

Colonies of spontaneously hypertensive rats of the stroke-prone strain (SHRSP) (Okamoto et al., 1974) and normotensive Wistar Kyoto rats (WKY) as well as Brattleboro rats homozygous for hypothalamic diabetes insipidus (DI) (Valtin and Schröder, 1964) and Long-Evans rats (LE) from which DI rats originally derived, have been bred at the Department of Pharmacology, University of Heidelberg, since 1975 (Dietz et al., 1978; Möhring et al., 1974).

SHRSP rats in the 14th generation were crossbred with DI rats starting in 1978. The F-1 generation of this crossing is heterozygous (di/+) for the DI allele (di/di) and characterized by a "dilution" of SHR genes. The genetic strategy therefore was to try to accumulate SHR genes as well as DI alleles in a subline of the F-1 generation. This was achieved alternatively first by inbreeding of di/+ animals and then introduction of new SHR genes from the SHRSP stock colony. This procedure led to a stepwise accumulation of SHR genes and a re-appearance of DI animals from the di/+ x di/+ intercrosses (Fig. 1). Rats with diabetes insipidus and spontaneous hypertension were obtained in the F-7 generation.

To characterize the new line of SHRDI rats they were compared with the original SHRSP and normotensive control WKY as well as with original DI and control LE rats. Arterial pressure and heart rate were measured in conscious, freely moving rats. The response to an 8 hour period of water deprivation and a subcutaneous injection of 5 µg/kg 1-deamino-8-D-arginine vasopressin (DDAVP), a synthetic non-pressor analogue of AVP with minimal cardiovascular activity (Sawyer et al., 1979; Johnson et al., 1979) was investigated in all 5 different strains.

Blood was collected from the retroorbital venous plexus under light ether anaesthesia. Hematocrit was determined by means of the microcapillary technique, plasma osmolality by freezing point depression (Knauer Osmometer). Fluid and food intake as well as urine volume and urine osmolality were measured in rats placed into metabolic cages.

The radioimmunoassays for arginine vasopressin (AVP) and oxytocin (OXT) have been described elsewhere (Rascher et al., 1981a; Rascher et al., 1982; Lang et al., 1981b). The identity of the radioimmunological activity of AVP and OXT in various brain areas was demonstrated by high-pressure liquid chromatography (µ-Bonda-pak C-18, Waters, Königstein, F.R.G; 0.01 ammonium acetate buffer, pH 5.4, methanol gradient, 30-80%).

For immunocytochemistry male rats were fixed by retrograde aortic perfusion with a solution of 0.1% glutaraldehyde in formaldehyde-picric-acid (50%) of a saturated picric-acid solution containing 18.5% formaldehyde. The brains of the animals were embedded in paraffin and cut in 7 µm thick sections. These paraffin sections. These paraffin sections were stained by using the peroxidase-anti-peroxidase technique (PAP) (Swanson, 1977). Specificity tests were carried out by preabsorbing the appropriate antibodies with AVP or OXT, and by using preimmune sera instead of antisera.

All results are given as means ± SEM. Significance of differences was assessed by analysis of variance. In those cases in which a difference of statistical significance was obtained, Scheffé's test was used for further evaluation.

Water intake and urine output were more than 4 times higher in SHRDI and DI rats as found in WKY, SHRSP and LE rats (Table 1). Mean arterial pressure was markedly elevated in SHRSP as well as in SHRDI rats, but heart rate did not differ in the 5 different strains of rats tested. AVP was not measurable in plasma and brain of SHRDI and DI rats (Table 1).

The OXT content in the pituitary and hypothalamus was reduced in SHRDI and DI

Table 1

Characteristics of SHRDI rats in comparison to appropriate controls.

	WKY	SHRSP	SHRDI	DI	LE
n	10	10	7	8	8
water intake (ml/24 h)	33.3 $\pm$ 1.1	37.1 $\pm$ 2.7	137 $\pm$ 6.5	125 $\pm$ 10.5	35.1 $\pm$ 3.1
mean arterial pressure (mmHg)	117 $\pm$ 2.7	197 $\pm$ 5.2*	184 $\pm$ 9.7*	101 $\pm$ 2.7	103 $\pm$ 2.1
heart rate (bpm)	340 $\pm$ 13	336 $\pm$ 8	352 $\pm$ 14	348 $\pm$ 16	345 $\pm$ 13
plasma AVP (fmol/ml)	2.77 $\pm$ 0.43	5.05 $\pm$ 0.73	0	0	3.51 $\pm$ 0.31
hypothalamus AVP (pmol/mg protein)	59.8 $\pm$ 2.6	34.9 $\pm$ 3.0	0	0	44.9 $\pm$ 6.6
pituitary AVP (nmol/mg protein)	1.59 $\pm$ 0.13	1.77 $\pm$ 0.16	0	0	1.45 $\pm$ 0.10
hypothalamus OXT (pmol/mg protein)	26.4 $\pm$ 2.8	15.8 $\pm$ 2.2	3.8 $\pm$ 0.9	10.4 $\pm$ 1.2	23.2 $\pm$ 1.8
pituitary OXT (nmol/mg protein)	1.95 $\pm$ 0.22	1.54 $\pm$ 0.14	0.17 $\pm$ 0.06	0.70 $\pm$ 0.10	1.74 $\pm$ 0.02

* p < 0.001

Table 2

Volume homeostasis in 5 months old SHRDI rats compared with age-matched control groups

	WKY	SHRSP	SHRDI	DI	LE
n	6	6	6	6	6
body weight (g)	306 $\pm$ 8.5*	222 $\pm$ 3.1	194 $\pm$ 9.0	216 $\pm$ 12.2	234 $\pm$ 6.7
hematocrit (%)	48.2 $\pm$ 0.6	50.2 $\pm$ 0.5++	52.2 $\pm$ 1.0++	44.2 $\pm$ 1.1	44.6 $\pm$ 0.7
plasma osmolality (mosmol/kg of water)	297 $\pm$ 3.1	292 $\pm$ 1.6	307 $\pm$ 4.0	307 $\pm$ 3.4	297 $\pm$ 4.4
water intake (ml/24 h)	13 $\pm$ 2.2	18 $\pm$ 3.0	151 $\pm$ 22*	122 $\pm$ 12*	11 $\pm$ 0.9
urine volume (ml/24 h)	11.5 $\pm$ 1.3	11.2 $\pm$ 1.5	123 $\pm$ 17*	113 $\pm$ 12*	9.5 $\pm$ 0.7
urinary osmolality (mosmol/kg of water)	2026 $\pm$ 279	1695 $\pm$ 198	190 $\pm$ 33*	262 $\pm$ 26*	2195 $\pm$ 68

* p < 0.01 compared with the other groups

+ p < 0.001 compared with the other groups

++ p < 0.01 compared with DI and LE rats

Table 3Response to subcutaneous injection of 5 μ g/kg 1-deamino-8-D-arginine vasopressin (DDAVP) in SHRDI rats compared with various age-matched control groups.

	WKY	SHRSP	SHRDI	DI	LE
n	6	6	6	6	6
urine volume (ml/8 h)	2.2 $\pm$ 0.5	2.0 $\pm$ 0.3	3.0 $\pm$ 0.6	3.8 $\pm$ 0.8	2.3 $\pm$ 0.3
urine osmolality (mosmol/kg of water)	2877 $\pm$ 337	3080 $\pm$ 87	976 $\pm$ 65*	717 $\pm$ 88 *	2865 $\pm$ 150

* p < 0.01 compared with the other groups

rats as compared to WKY, SHRSP and LE rats (Table 1). Water intake and output of urine were the same in SHRDI and DI rats (Table 2). Urine osmolality was markedly reduced. During an 8 hour period of water deprivation SHRDI and DI rats were unable to concentrate their urine adequately. However, SHRDI and DI rats did concentrate their urine 3 to 4 fold after subcutaneous administration of DDAVP (Table 3). In all animals positive immunostaining was observed in the hypothalamus for oxytocin. AVP immunocytochemistry was negative in DI and SHRDI rats.

The successful breeding of a new line of SHR rats with hereditary hypothalamic diabetes insipidus and spontaneous hypertension solves the controversy whether AVP is necessary for the development and maintenance of high blood pressure in spontaneous hypertension of rats or not. It is obviously not.

In SHRDI rats blood pressure is similarly elevated as in SHRSP rats of the same age. The reduction of urine volume and the increase in urine osmolality to exogenously applied DDAVP in SHRDI and in DI rats demonstrate that the kidney tubules respond normally to vasopressin and that the defect in the ability to concentrate urine in SHRDI is not the result of an acquired kidney disease; indeed, lack of hypothalamic AVP synthesis is responsible for the distinct disturbances of water balance in these rats. Using bioassay methods it had been shown previously that pituitary OXT synthesis is intact but that the content of OXT is about one third in DI rats compared with that of LE rats (Valtin et al., 1965). This finding is confirmed in the present experiments in which pituitary OXT was measured radioimmunologically. In the hypothalamus of DI rats, OXT content was only one half that found in LE rats. Similar as in DI, SHRDI rats also had reduced contents of OXT in the hypothalamus and pituitary gland when compared to WKY or LE rats. Further neuroendocrine parameters need to be studied.

The data available so far on SHRDI rats indicate that they are endocrinologically similar or identical to DI rats. This facilitated the selection of the new strain during the breeding.

The results of these experiments allow two conclusions. Firstly, that spontaneous hypertension in rats can develop in the absence of AVP. This solves a long-standing controversy (Möhring et al., 1980; Johnston et al., 1981; Rascher et al., 1981a; Rascher et al., 1982; Lang et al., 1981b). In view of the depressor effects of brain AVP (Versteeg et al., 1979; Rascher et al., 1981b) this genetic defect may even facilitate the establishment of high blood pressure. Secondly, breeding of animals with selective defects in gene expression is a promising new way to study complex pathophysiological mechanisms such as the multigenetic hypertensive disease by dissecting out one single factor. This approach which can involve genetic selection by breeding as in these experiments or new methods in gene technology, opens up new possibilities in the study of multigenetic diseases.

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