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DIFFERENCE BETWEEN MALE AND FEMALE RATS IN VASOPRESSOR RESPONSE TO ARGININE VASOPRESSIN+

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A study was carried out how the sexual difference influences the increase in blood pressure (BP) induced by arginine vasopressin (AVP), and how the binding characteristics of ^{3}H-labelled AVP on membranes prepared from the vascular bed were affected. After the administration of various doses of AVP, a significantly higher BP increase was observed in male rats than in females. The vasopressor effect of AVP was reduced in males following orchidectomy or administration of the antiandrogen cyproterone acetate. The vasopressin (VP) antagonist d(CH_2)$_5$Tyr(Me)AVP diminished the BP response to AVP in both sexes. The plasma AVP level was found to be much higher in males than in females, but it was decreased to the level of females after orchidectomy. The density of AVP-binding sites in the aorta membrane preparation was smaller in females, and in orchidectomized or cyproterone acetate-treated male rats than in the control males.

The results demonstrate that testosterone upregulates the number of AVP-binding sites, leading to an increase in the pressor response to AVP in the rat vascular bed.

Keywords: blood pressure, AVP, V_1 antagonist, orchidectomy, cyproterone acetate, aorta membrane preparation, AVP binding site

Sexual hormones have been shown to be involved in regulation of the biological action of vasoactive agents [2, 14]. We previously demonstrated that VP can induce a considerable renal vasospasm in rats treated with oestrogen [5, 8], and renal cortical necrosis can develop as a consequence of the vasoconstriction [7, 14].

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The renal arteries can be sensitized by oestrogen against the vasoconstrictive action of VP. A similar sensitizing effect was observed in rats treated with testosterone, but the renal cortical necrosis was more pronounced following VP administration in testosterone-pretreated rats than in rats treated with oestrogen. In the present work, a study was made of how sexual difference influences the increase in BP induced by AVP, and the binding characteristics of ^{3}H-labelled AVP on membranes prepared from the rat aorta. In the third experimental series, the effects of the VP antagonist d(CH_2)$_5$Tyr(Me)-AVP on the BP increase produced in male and female rats were examined.

Materials and Methods

The experiments were carried out on Wistar rats of the same age (12–14 weeks old), the females weighing 180–220 g and the males 250–300 g. The animals were anaesthetized with pentobarbital, administered i.p. in a dose of 45 mg/kg body weight (b.w.). After the anaesthetization, a cannula was inserted into the trachea, the left common carotid artery was prepared in the neck, the vagal nerve was separated from the vessel, and 0.1 ml physiological saline containing 5 U heparin was administered via a polyethylene cannula (outer diameter/inner diameter 0.8/0.5 mm) inserted into the carotid lumen, to inhibit blood clotting. The cannula was connected to a Hellige electromanometer, and the BP was recorded on a Biocomp five-channel polyphysiograph. Following the cervical preparation, phentolamine (Regitin, Ciba-Geigy) was administered i.p. in a dose of 10 mg/kg b.w. AVP (376 IU/mg) (0.02, 0.06, 0.18 μg/kg b.w.) was injected in a logarithmically increasing dose through a small syringe fixed into the lateral tail vein.

The effect on the BP was always measured continuously after AVP administration, and the BP increase was expressed as a percentage of the initial value. The VP antagonist [1-(β-mercapto-ββ-cyclopentamethylenepropionic acid)-2-O-methyltyrosine]AVP, d(CH_2)$_5$Tyr(Me)AVP, was injected in a dose of 0.2 μg/kg into the lateral tail vein 20 min before AVP administration. The dose of the antagonist was calculated as an effective antipressor dose (0.16 nmol/kg = 184 ng/kg), defined as the quantity which halves the pressor effect observed 20 min after i.v. administration of 2.0 IU AVP [9]. The method of BP measurement and detection of the effectiveness of the VP antagonist on the BP were described in detail in an earlier study [15].

Bilateral orchidectomy was carried out under ether anaesthesia 10 days before the experimentation. The efficiency of orchidectomy was controlled via the measurement of plasma testosterone (RIA). The antiandrogen cyproterone acetate (Androcur, Schering) was administered through a gastric tube in an oral dose of 2.5 mg/day once daily for 10 days. Testosterone (Retandrol, Richter) was injected s.c. in a dose of 2.5 mg/day once daily for 10 days.

Tissue preparation: Rat aorta plasma membrane was prepared by a two-step centrifugation procedure, according to the method of Pearlmutter et al. [19] with some modifications. The rats were decapitated, and the aorta was removed with forceps and placed in homogenizing buffer (100 mM TRIS/HCl, Ph 7.4, 10 mM $MgCl_2$). The tissue was cut into small pieces and homogenized with a teflon homogenizator. The liquid suspension was centrifuged at 1000 × g for 10 min at 4 °C. The supernatant was centrifuged at 100,000 × g for 60 min. The pellett was resuspended in homogenizing buffer and stored at −70 °C until further use.

Binding assay: A membrane suspension (containing 100 μg protein) in 100 ml binding buffer (50 mM TRIS/HCl, pH 7.4, 5 mM $MgCl_2$) containing 10 nM ^{3}H-AVP in the presence of various

concentrations of unlabelled AVP was incubated at 30 °C for 30 min. To terminate the binding tests, the mixtures were diluted with 5 ml ice-cold washing buffer (10 mM TRIS/HCl, pH 7.4, 2 mM $MgCl_2$ and 0.05% BSA), immediately filtered under vacuum through Whatman GF/F filters, and rinsed twice with 5 ml ice-cold washing buffer. The filters were placed into counting vials with scintillation liquid and analysed by liquid scintillation spectrometry. Protein was determined by means of a modified fluorescamine assay [24]. B_{max} and K_D values were determined by the method of Fahrenholz et al. [3]: Binding curves were fitted to a logistic function with a weighed iterative least-squares procedure based on the method of steepest descent.

Biometric analysis was carried out with the two-tailed Student test.

Results

Following the administration of various doses of AVP, a significantly higher BP increase was observed in males than in females (Fig. 1). After injection of the V1 antagonist $d(CH_2)_5Tyr(Me)AVP$ in a dose of 0.06 μg/kg b.w., the pressor response to AVP was substantially reduced in both sexes, but the reduction was more pronounced in males than in females. The effects of testosterone, orchidectomy and cyproterone acetate administration on the BP changes induced by AVP are demonstrated in Fig. 2. The reactivity of the vascular bed to AVP in male rats was not enhanced by the administration of testosterone. The vasopressor effect of AVP was less pronounced 10 days after bilateral orchidectomy. The BP response to AVP in males was also reduced after administration of the antiandrogen cyproterone acetate. In this case the pressor activity of AVP was the same in the two sexes. The plasma AVP level proved much higher in males than in females (Fig. 3). Following

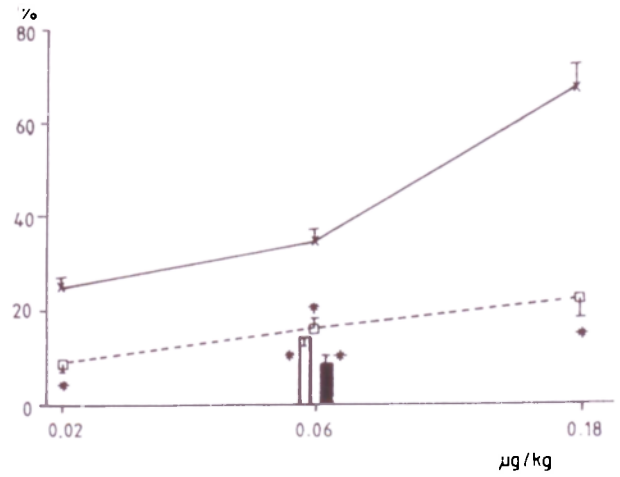

Fig. 1. Percentage changes in BP in male and female rats following VP and/or V_1 antagonist administration. X: male, VP (n:24); □: female, VP (n:25); ■: male, VP + V_1 antagonist (n:24); ▪: female, VP + V_1 antagonist (n:25). Mean ± S. E. M. *: significant difference

orchidectomy, the AVP concentration was reduced to the level of females. Cyproterone acetate treatment did not influence the AVP level in males. The plasma testosterone level was diminished by orchidectomy and remained unchanged after cyproterone acetate treatment.

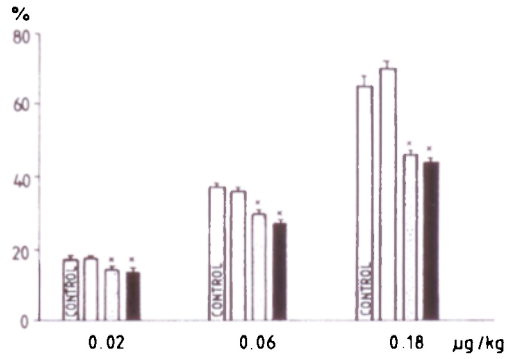

Fig. 2. Percentage changes in BP following VP administration in male rats treated with testosterone, cyproterone acetate or orchidectomy. ⧄: untreated control (n:12); □: testosterone treatment (n:10); ⧄: orchidectomy (n:12); ▬: cyproterone acetate treatment (n:11). Mean ± S. E. M. *: significant difference

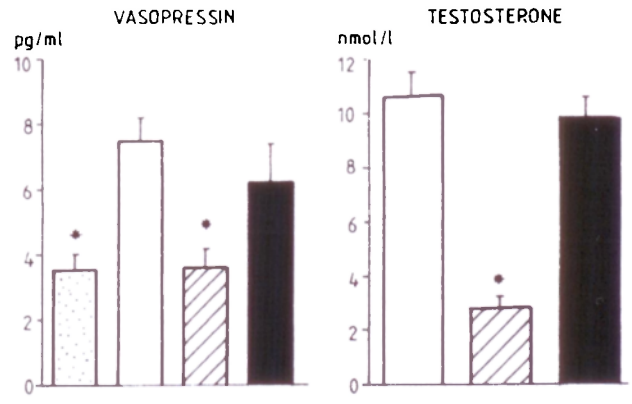

Fig. 3. Plasma AVP and testosterone levels following orchidectomy or cyproterone acetate treatment. ⧄: untreated female (n:20); □: untreated control male (n:20); ▨: orchidectomy (n:12); ■: cyproterone acetate treatment (n:11). Mean ± S. E. M. *: significant difference vs. control

In comparison with females or with orchidectomized or cyproterone acetate-treated males, male rats displayed an increase in the density of AVP-binding sites in the aorta membrane preparation (Table I). The maximum observed binding capacity (B_{max}) of ^{3}H-AVP was significantly smaller in females, and in orchidectomized or cyproterone acetate-treated male rats than in control males. The ligand affinity of

untreated male rats proved to be moderately reduced in comparison with the other goups. The experiment on the specific binding of ^{3}H-AVP resulted in a much flatter saturation curve for female (Fig. 4) than for male (Fig. 5) rat aorta crude membranes.

Table I

Specific ^{3}H-AVP binding to rat aorta membranes in different groups

Group	No. of animals	Maximal binding capacity (B_{max}) fmol/mg protein	Ligand concentration (K_D) nM
1. Controls, male	16	115 ± 14.5+	5.7 ± 0.7
2. Controls, female	16	65 ± 6.0*	3.6 ± 0.3*
3. Orchidectomized, male	14	77 ± 8.0*	3.8 ± 0.2*
4. Cyproterone acetate treatment, male	15	80 ± 8.5*	2.9 ± 0.6*

mean ± S. E. M.
*: significant difference vs. control males

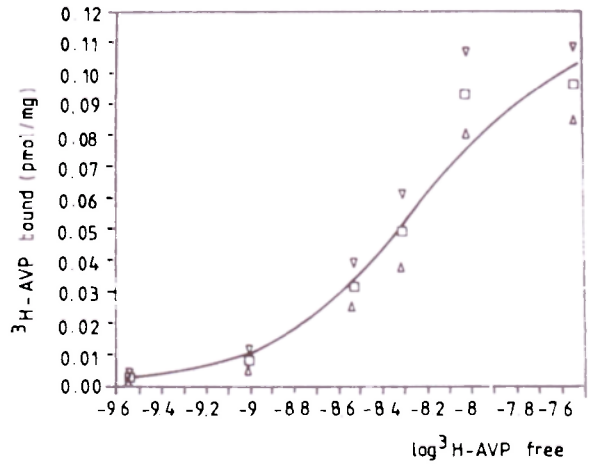

Fig. 4. Saturation curve of the specific binding of ^{3}H-AVP to male rat aorta crude membranes

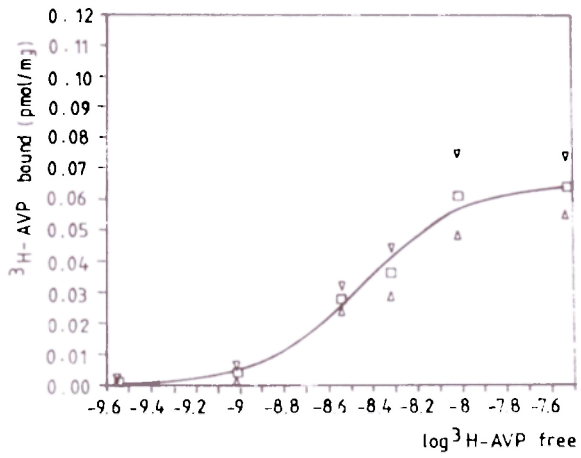

Fig. 5. Saturation curve of the specific binding of ^{3}H-AVP to female rat aorta crude membranes

Discussion

This investigation demonstrates that different doses of AVP induce a significantly higher BP in males; the vasopressor effect of AVP is less pronounced in male rats after orchidectomy and following administration of the antiandrogen cyproterone acetate; the density of AVP-binding sites in the aorta membrane preparation was found to be lower in females, and in orchidectomized or cyproterone acetate-treated male rats than in the control males. However, the ligand affinity was moderately smaller in untreated control male group.

We earlier observed a similar phenomenon in connection with renal vessels. VP administration after testosterone pretreatment induced renal cortical necrosis in rats, the histological picture pointing to the hypoxic origin of the change [18]. Testosterone sensitizes the renal arteries to the vasoconstrictive effect of VP [6]; renal cortical necrosis cannot be observed following the injection of VP alone, without testosterone pretreatment. Cyproterone acetate prevented the sensitizing effect of testosterone: as a result of the administration of cyproterone acetate simultaneously with testosterone, the subsequent administration of a large dose of VP did not result in renal cortical necrosis. This circumstance permits the conclusion that the sensitizing effect of testosterone is manifested through the mediation of the androgen receptors [6, 18]. This assumption is supported by observations proving the presence of androgen receptors in the kidney [1, 16, 22, 25].

The sensitizing effect of testosterone has also been detected in another region of the blood supply. In an earlier study we demonstrated that endogenous VP is of great importance in the pathogenesis of gastric haemorrhagic lesions induced by a

high concentration of ethanol [12, 13]. Recent observations revealed that ethanol generates more severe lesions in the gastric mucosa of male rats than in females. Orchidectomy and cyproterone acetate treatment each reduced the extent of ethanol-induced gastric erosions in male rats [10, 11].

The present results can be compared with findings of investigations of the relationship between the plasma testosterone level and the number of kidney AVP receptors in aged rats. A decreased number of renal binding sites for AVP in the aged rat was demonstrated both by a membrane-binding assay [4, 17] and by an immunocytochemical staining procedure [20]. An increasing functional impairment of the hypothalamo-neurohypophyseal system with aging was described by Turkington and Everitt [23], Sladek et al. [21] and Zbuzek et al. [26]. At the same time, the plasma testosterone concentration was found to be reduced in aged rats [4, 20], and testosterone treatment increased the number of AVP-binding sites in the aged kidney [4].

In conclusion, the vasopressor effect of AVP has been shown to be more pronounced in male rats than in females. After orchidectomy or the administration of cyproterone acetate, the BP increase induced by AVP in males was reduced to the level of females. This sensitizing effect of testosterone is developed via an increase in the AVP-binding sites found in the walls of the vascular beds.

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