J. Steroid Biochem. Molec. Biol. Vol. 40, No. 4–6, pp. 511–515, 1991
Printed in Great Britain

0960-0760/91 $3.00 + 0.00
Pergamon Press plc

SEXUAL DIMORPHISM OF BLOOD PRESSURE: POSSIBLE ROLE OF THE RENIN–ANGIOTENSIN SYSTEM

JÜRGEN BACHMANN, MONIKA FELDMER, URSULA GANTEN, GÜNTER STOCK and DETLEV GANTEN*

German Institute for High Blood Pressure Research and Department of Pharmacology, University of Heidelberg, Im Neuenheimer Feld 366, 6900 Heidelberg, Fed. Rep. Germany

Summary—The prevalence of hypertension in men is higher than in women and the onset of this disease is earlier in male than in female subjects. In spontaneously hypertensive rats, males also have higher blood pressures than females. Evidence from epidemiological, physiological, molecular biological and morphological studies concerning this sexual dimorphism is reviewed. We demonstrate that the gonadal steroids testosterone and estrogen have important effects on the gene regulation of the renin–angiotensin system. This may in part contribute to the sexual dimorphism in blood pressure control. The direct effect of steroid hormones on genes related to hypertension provides a suitable paradigm to improve our understanding of molecular and cellular mechanisms of cardiovascular control.

INTRODUCTION

The higher prevalence of hypertension in men has been confirmed in several epidemiological studies [1–3]. The Framingham 18 Year Follow-up Study has shown that there is a gender discrepancy in the prevalence of hypertension in humans with 33% hypertension in U.S. men aged 18–79 years compared to 27% in women of comparable age. Higher blood pressure levels in males than in females have also consistently been reported in genetically hypertensive rats and mice [4, 5] and in deoxycorticosterone(DOC)-salt hypertension [6], a form of hypertension that requires the presence of vasopressin [7]. The underlying mechanisms for the sexual dimorphism of blood pressure in hypertension are not completely understood, although there is much evidence for an important role of gonadal steroids in cardiovascular control mechanisms. Evidence will be reviewed about the participation of testosterone and estrogens in cardiovascular regulation and the possible role of the renin–angiotensin system.

TESTOSTERONE

Sexual brain differentiation is sensitive to the neonatal gonadal hormone pattern. Cambotti *et al.* [4] and Ganten *et al.* [5] showed that the neonatal gonadal hormonal milieu influences the sexual dimorphic pattern of blood pressure in spontaneously hypertensive rats (SHR). Testosterone treatment of neonatal female SHR resulted in an elevation of blood pressure in later life which reached the same level as that of male rats of comparable age. Chemical or surgical castration of neonatal male rats on the other hand led to a marked attenuation of blood pressure in later life. Blood pressure in these rats did not differ significantly from female rats of the same age. When exposed to testosterone in adulthood, blood pressure increased more in neonatally testosterone treated female rats than in control females, suggesting that neonatal sexual differentiation of brain areas concerned with the control of blood pressure influences the sensitivity of blood pressure to testosterone in later life [4].

The effect of testosterone on blood pressure development in SHR is not restricted to the neonatal period, since blood pressure was also reduced in 9-week-old SHRSP which were either orchidectomized or treated with the testosterone receptor antagonists flutamide or cyproterone acetate. These data suggest that,

Proceedings of the VIIIth International Congress on Hormonal Steroids, The Hague, The Netherlands, 16–21 September 1990.

*To whom correspondence should be addressed.

in addition to brain maturation, testosterone affects blood pressure development in male genetically hypertensive rats via direct hormone receptor-mediated mechanisms. Chemical or surgical castration in adult rats at 25 weeks of age did not influence blood pressure, however, indicating that in chronic hypertension, testosterone receptor-mediated mechanisms are not directly involved in the maintenance of high blood pressure. The underlying mechanisms of how testosterone influences blood pressure are not understood. Cunard *et al.* [8] found that the vascular endothelium of male rats has a lower relaxing potency and releases lower levels of vasodilator substances than the endothelium of female rats. In the wall of large arteries, receptors for estradiol, dihydrotestosterone and corticosteroids have been identified [9]. It is well established that testosterone interacts with hormonal systems that are directly involved in the control of blood pressure and volume homeostasis. Lara *et al.* [10] reported that testosterone modulates norepinephrine storage and release in sympathetic fibers innervating vas deferens. Autoradiographic studies with [^{3}H]dihydrotestosterone have shown that neural nuclear labeling is present within brain areas that are involved in the control of cardiovascular function such as in the rat spinal cord, in the lower brain stem, in the area postrema, dorsal motor nucleus of the vagus and nucleus ambiguus, in the region of the catecholamine group A5, raphe nuclei, in the central gray of the midbrain, basal hypothalamus, periventricular nucleus, preoptic region, bed nucleus of the stria terminalis, dorsolateral septum, and amygdala [11]. This shows that androgen binding sites are present in the area postrema and in the preoptic region, which are thought to be important for the control of blood pressure and heart rate [12–15]. Testosterone may therefore directly affect production of peptides with cardiovascular effects in the anterior hypothalamus and in the caudal medulla. Dihydrotestosterone and catecholamine co-localization has been found in 50–80% of catecholamine neurons in the pons and the dorsolateral corner of the 4th ventricle, adjacent to the n. olivaris superior, in the locus coeruleus, the region of the lemniscus lateralis and in the n. arcuatus and n. periventricularis hypothalami [16].

Testosterone has been shown to also enhance the pressor responsiveness to vasopressin. After castration DOC-salt hypertension is attenuated [6]. The renin–angiotensin system is also affected by testosterone. Renin content and renin mRNA in the salivary gland of mice are responsive to androgens [17, 18]. Data from our laboratory showed that dihydrotestosterone treatment of NMRI mice enhanced renin mRNA levels in the brain, submandibular gland, adrenal gland and heart [19], which may reflect an important mechanism of the blood pressure increasing effect of dihydrotestosterone. Interestingly, there was an early rise of renin mRNA levels 2 h after testosterone treatment in the adrenal gland, whereas in the brain, a significant elevation of renin mRNA was measured after a treatment of 21 days. A possible explanation for this observation is that there are tissue specific responses of the local renin–angiotensin systems to testosterone and that different tissues participate in a differentiated manner with respect to time, mechanism and magnitude of renin stimulation.

ESTROGENS

There are conflicting reports concerning the effect of estrogens on the cardiovascular system. On the one hand, it has been demonstrated that the treatment of SHRSP with estradiol inhibited the development of hypertension [20]. On the other hand, it is known that the prevalence of hypertension is higher in pregnancy. Use of oral contraceptives has also been reported to elevate blood pressure. Estrogens interact with several hormonal systems that are involved in cardiovascular regulation. Urinary vasopressin excretion in rats, for example, is sex-related, males excreting more vasopressin than females. This difference was abolished by gonadectomy. Treatment of ovariectomized females with estradiol, progesterone, or estradiol plus progesterone as well as substitution of castrated males with testosterone restored the urinary excretion of vasopressin to normal levels. Pressure responsiveness to vasopressin was greater in male rats than in female rats [6]. Pretreatment of rats with estrogen induced enhanced constrictor actions of mesenteric arterioles to oxytocin, vasopressin and catecholamines [21].

Estrogen binding sites have been found in brain regions that have been implicated in the regulation of the cardiovascular system [22]. Estrogen target neurons in the spinal cord include the nucleus intermediolateralis. In the brain stem, estrogen target neurons are found in the nuclei of the solitary tract, dorsal motor nucleus of the vagus, nucleus ambiguus, ventro-

lateral reticular formation, raphe nuclei, central gray, nuclei in the basal hypothalamus, paraventricular and periventricular nuclei in the anterior hypothalamus, preoptic region, lateral septum, bed nucleus of the stria terminalis and nuclei in the amygdala [23]. Between 50 and 80% of the estrogen target neurons contain catecholamine fluorescence in the regions of the nucleus reticularis lateralis, n. tractus solitarii, pons, locus coeruleus and in the vicinity of the lemniscus lateralis [16]. A small number of dopamine neurons in the hypothalamic arcuate and periventricular nucleus are labeled with estradiol. Co-localization is also found with serotoninergic neurons in various raphe nuclei, with neurotensin containing neurons in the n. tractus solitarii, oxytocin in the anterior hypothalamus, endorphin and enkephalin in the arcuate nucleus and somatostatin, GABA, and atrial natriuretic peptide in the periventricular hypothalamus. In the heart, co-localization between ANF and estradiol has been described (for a review see Ref. [9]).

Estrogens also interact with the renin–angiotensin system. Chronic estradiol treatment decreases angiotensin II receptor density in the adrenal cortex and in the pituitary gland which may indicate that estradiol modulates responsiveness of different cell types to angiotensin II in some tissues through receptor down-regulation [24]. Angiotensinogen gene expression is regulated by estrogens *in vivo*. Studies in isolated hepatocytes and hepatoma cells derived from humans and rats showed that estrogens stimulated angiotensinogen synthesis 2-fold [25, 26]. In our laboratory a sequence with strong homology to known estrogen responsive elements was identified in the 5′ flanking region of the rat angiotensinogen gene. In the absence of this sequence, the gene construct that consisted of the 5′ flanking region of the rat angiotensinogen gene and the bacterial chloramphenicol acetyltransferase gene (CAT) was not inducible by estrogens, suggesting that this region functions as an estrogen responsive element [27]. In the 5′ flanking region of the human angiotensinogen gene, an estrogen responsive element was equally identified [28]. These data suggest that the regulation of the rat angiotensinogen gene by estrogens takes place

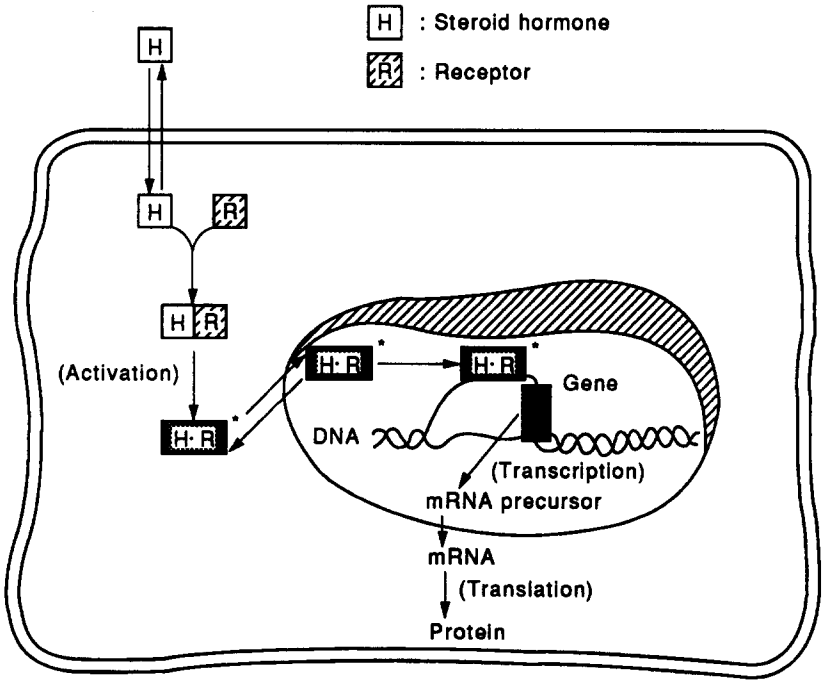

Fig. 1. Schematic illustration of mechanisms by which steroid hormones regulate the activity of specific genes. Steroid hormones (H) enter the cell freely. In the cytosol they can be metabolized by specific enzymes or bind directly to their respective receptors (R). The steroid receptor complex (H·R) is then transferred into the nucleus where it interacts directly with DNA to initiate transcription of steroid responsive genes. This model could partially explain the activation of the renin–angiotensin system by steroid hormones. For more detailed discussion see Refs [5, 19].

at a transcriptional level and that the interaction of the steroid hormone receptors with the gene is conferred by discrete regulatory elements in the 5′ flanking region of the angiotensinogen gene. These results contribute to a better understanding of how estrogens regulate the rat angiotensinogen gene on the molecular level. These mechanisms may be involved in the rise of plasma angiotensinogen levels after treatment with estrogens and during pregnancy [29–32] and possibly in steroid induced hypertension.

CONCLUSION

To date, there is no conclusive explanation for the sexual dimorphism with respect to blood pressure in animals and in man. Future research that is directed towards the molecular mechanisms of the interaction of gonadal steroids with genes that are important for cardiovascular regulation will provide new insights into the mechanisms which contribute to this phenomenon. Interestingly, the transgenic rats TGR(mRen2)-27 harbouring an additional renin gene exhibit higher blood pressure in male as compared to female with a difference in adult animals of about 100 mmHg [32]. This supports the concept of the renin gene being involved in these blood pressure differences. We have discussed above that testosterone can stimulate renin mRNA in mice. It is this DAB/ren2d mouse gene which was introduced into the transgenic rats and found to produce fulminant hypertension. It is therefore tempting to speculate that the renin gene and the stimulatory effects of testosterone on its expression [19] are in part responsible for the higher blood pressure in male compared to female subjects. Other components of the renin–angiotensin system, e.g. angiotensinogen, may be involved as well in pathophysiological conditions associated with hypertension.

As discussed above, modulating effects of steroid hormones on brain function, endothelial paracrine activity and sexual differences in the complex pressor and depressor systems need to be considered. Since gonadal steroid hormones have direct effects on cellular function and gene expression (Fig. 1), a better understanding of the mechanisms responsible for the differences in blood pressure and cardiovascular risk between women and men, could help to elucidate the basic mechanisms of primary, genetic hypertension and improve the development of selective therapeutic strategies.

REFERENCES

1. Keil U., Remmers A., Chambless L., Hense H. W., Stieber J. and Lauck A.: Epidemiologie des Bluthochdrucks. *Münch. Med. Wschr.* **23** (1986) 424–429.
2. Dawber T. R.: *The Framingham Study. The Epidemiology of Atherosclerotic Disease.* Harvard University Press, Cambridge, MA (1980).
3. HDFP Cooperative Group: Race, education and prevalence of hypertension. *Am. J. Epidem.* **106** (1977) 351–361.
4. Cambotti L. J., Cole F. E., Gerall A. A., Frohlich E. D. and MacPhee A. A.: Neonatal gonadal hormones and blood pressure in the spontaneously hypertensive rat. *Am. J. Physiol.* **247** (1984) 258–264.
5. Ganten U., Schröder G., Witt M., Zimmermann F., Ganten D. and Stock G.: Sexual dimorphism of blood pressure in spontaneously hypertensive rats: effects of anti-androgen treatment. *J. Hypert.* **7** (1989) 721–726.
6. Share L., Crofton J. T. and Ouchi Y.: Vasopressin: sexual dimorphism in secretion, cardiovascular actions and hypertension. *Am. J. Med. Sci.* **31** (1988) 314–319.
7. Crofton J. T., Share L., Shade R. E., Lee-Kwon W. J., Manning M. and Sawyer W. H.: The importance of vasopressin in the development and maintenance of DOC-salt hypertension in the rat. *Hypertension* **1** (1979) 31–38.
8. Cunard C., Falcon J., Maddox Y., Riniger M. and Ramwell P.: Eicosanoid expression of vascular sexual dimorphism. In *Prostacyclin and its Stable Analogue Iloprost* (Edited by R. J. Gryglewski and G. Stock). Springer, Berlin (1987) pp. 115–122.
9. Stumpf W. E.: Steroid hormones and the cardiovascular system: direct actions of estradiol, progesterone, testosterone, gluco- and mineralocorticoids, and soltriol (vitamin D) on central nervous regulatory and peripheral tissues. *Experientia* **46** (1990) 13–25.
10. Lara H., Galleguillos X., Arran J. and Belmar J.: Effects of castration and testosterone on norepinephrine storage and on the release of [^{3}H]norepinephrine from rat vas deferens. *Neurochem. Int.* **7** (1985) 667–674.
11. Sar M. and Stumpf W. E.: Distribution of androgen-concentrating neurons in rat brain. In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 120–133.
12. Buggy J. and Fisher A. E.: Anteroventral third ventricle site of action for angiotensin induced thirst. *Pharmac. Biochem. Behav.* **4** (1976) 651–660.
13. Diz D. I. and Jacobowitz D. M.: Cardiovascular actions of four neuropeptides in the rat hypothalamus. *Clin. Exp. Hypert.* **A6** (1984) 2085–2090.
14. Mangiapane M. L., Skoog K. M., Rittenhouse P., Blair M. L. and Sladek C. D.: Lesions of the area postrema region attenuates hypertension in spontaneously hypertensive rats. *Circ. Res.* **64** (1989) 129–135.
15. Skoog K. M. and Mangiapane M. L.: Area postrema and cardiovascular regulation in rats. *Am. J. Physiol.* **254** (1988) H963–H969.
16. Heritage A. S., Stumpf W. E., Sar M. and Grant L. D.: Brainstem catecholamine neurons as target sites for sex steroid hormones. *Science* **207** (1980) 1377–1379.
17. Wilson C. M., Cherry M., Taylor B. A. and Wilson J. D.: Genetic and endocrine control of renin activity in the submaxillary gland of the mouse. *Biochem. Genet.* **19** (1981) 509–523.
18. Catanzaro D. F., Mesterovic N. and Morris B. J.: Studies on the regulation of mouse renin gene by measurement of renin messenger ribonucleic acid. *Endocrinology* **117** (1985) 872–878.
19. Metzger R., Wagner D., Takahashi S., Suzuki F., Lindpaintner K. and Ganten D.: Tissue renin-angiotensin systems: aspects of molecular biology and pharmacology. *Clin. Exp. Hypert.* **A10** (1988) 1227–1238.

20. von Eiff A. W., Lutz H. M., Gries J. and Kretschmer R.: The protective mechanism of estrogen on high blood pressure. *Bas. Res. Card.* **80** (1985) 191–202.
21. Altura B. M.: Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. *J. Pharmac. Exp. Ther.* **193** (1975) 403–412.
22. Stumpf W. E.: Estrogen-neurons and estrogen–neuron systems in the periventricular brain. *Am. J. Anat.* **129** (1970) 207–218.
23. Stumpf W. E., Sar M. and Keefer D. A.: Atlas of estrogen target cells in rat brain. In *Anatomical Neuroendocrinology* (Edited by W. E. Stumpf and L. D. Grant). Karger, Basel (1975) pp. 104–119.
24. Carriere P. D., De Lean A., Gutkowska J., Genest J. and Cantin M.: Chronic estradiol treatment decreases angiotensin II receptor density in the anterior pituitary gland and adrenal cortex but not in the mesenteric artery. *Neuroendocrinology* **43** (1986) 49–56.
25. Chang E. and Perlman A. J.: Multiple hormones regulate angiotensinogen messenger ribonucleic acid levels in a rat hepatoma cell line. *Endocrinology* **121** (1987) 513–519.
26. Kunapuli S. P., Benedict C. R. and Kumar A.: Tissue specific hormonal regulation of the rat angiotensinogen gene expression. *Archs Biochem. Biophys.* **254** (1987) 642–646.
27. Feldmer M., Kaling M., Takahashi S., Mullins J. J. and Ganten D.: Glucocorticoid and estrogen responsive elements in the 5′-flanking region of the rat angiotensinogen gene. *J. Hypert.* In press.
28. Clauser E., Gaillard I., Wei L. and Corvol P.: Regulation of angiotensinogen gene. *Am. J. Hypert.* 2 (1989) 403–410.
29. Eisenfeld A. J., Aten R. F. and Weinberger M. J.: Oral contraceptives—possible mediation of side effects via an estrogen receptor in liver. *Biochem. Pharmac.* **27** (1978) 2571–2575.
30. Dzau V. J. and Herrmann H. C.: Hormonal control of angiotensinogen production. *Life Sci.* **30** (1982) 577–584.
31. Hellmann W., Suzuki F., Ohkubo H., Nakanishi S., Ludwig G. and Ganten D.: Angiotensinogen gene expression in extrahepatic rat tissues: application of a solution hybridization assay. *Archs Pharmac.* **338** (1988) 327–331.
32. Mullins J. J., Peters J. and Ganten D.: Fulminant hypertension in transgenic rat harbouring the mouse Ren 2 gene. *Nature* **344** (1990) 541–544.