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NORADRENALINE CONCENTRATION AND TURNOVER IN NUCLEI OF THE HYPOTHALAMUS AND THE MEDULLA OBLONGATA AT TWO STAGES IN THE DEVELOPMENT OF RENAL HYPERTENSION IN THE RAT

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SUMMARY

The noradrenaline concentration and the α-methyl-para-tyrosine (α-MPT)-induced disappearance of noradrenaline were determined in several nuclei of the hypothalamus and the medulla oblongata of renal hypertensive rats (two-kidney Goldblatt hypertension). A decreased α-MPT-induced disappearance of noradrenaline was found in the nucleus interstitialis striae terminalis and the nucleus paraventricularis 3 days after renal artery constriction, when blood pressure was slightly, but significantly higher than that of sham operated rats. At this stage the α-MPT-induced disappearance of noradrenaline was enhanced in the nucleus commissuralis and the A_1-region of hypertensive rats while the noradrenaline concentration in the A_1-region was significantly elevated. No significant differences were found in both parameters in hypothalamic and medullary nuclei 3.5 weeks after the operation, when hypertension had fully developed. These findings are indicative of the occurrence of transient changes in the activity of noradrenergic neurons located in the medulla oblongata and projecting to the hypothalamus during the initiation of the development of two-kidney Goldblatt hypertension.

INTRODUCTION

Development of renal hypertension induced by application of a clip on an artery of one of the kidneys of rats with an intact contralateral kidney (two-kidney Goldblatt hypertension) is renin-dependent[4,6,9]. Part of the blood pressure elevation is probably exerted via peripheral actions of angiotensin, i.e. vasoconstriction and stimulation of

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aldosterone secretion by the adrenals[14]. Another site of action of angiotensin appears to involve the CNS. Angiotensin administered into the vertebral artery elevates the arterial pressure, whereas intracranial injection of this peptide induces drinking and a rise of blood pressure[5,17]. The possible relation between angiotensin and CNS catecholamines was demonstrated by Lewis et al.[10], who have shown that the hypertensive response to injections of angiotensin into the lateral ventricle was markedly reduced by intracisternal administration of 6-hydroxydopamine (6-OHDA). It is possible that the effect of angiotensin on the CNS contributes to the development of renal hypertension in the two-kidney Goldblatt model.

Petty and Reid[15,16] studied the activity of tyrosine hydroxylase (TH) and the level of noradrenaline in different brain regions of rats with one-kidney Goldblatt hypertension. In this form of renal hypertension the renin–angiotensin system appears to play a lesser role in the development of the hypertension[2,4]. Petty and Reid[15,16] reported transient changes in noradrenaline concentration and the activity of TH of nuclei in the anterior hypothalamus during the development of hypertension in the one-kidney Goldblatt rat. We found that in young spontaneously hypertensive rats (SH-rats), when compared to normotensive Wistar–Kyoto rats (WK-rats) of the same age, decreases are apparent in the α-methyl para-tyrosine(α-MPT)-induced disappearance of noradrenaline in a number of hypothalamic nuclei[23]. In older SH-rats with a more developed hypertension, the noradrenaline disappearance in the same brain regions has returned to normal values[23].

In the present communication we describe the results of experiments in which the noradrenaline concentration and the α-MPT-induced disappearance of noradrenaline were measured in nuclei of the hypothalamus and the medulla oblongata at an early and a later stage of the development of two-kidney Goldblatt hypertension.

METHODS

Animals

Male rats of an inbred Wistar strain were used (Wistar-Cpb.). Rats were obtained from the Centraal Proefdieren Bedrijf, T.N.O., Zeist (The Netherlands). Operations were performed under ether anesthesia. Renal hypertension (two-kidney Goldblatt hypertension) was induced by applying a solid silver clip with an internal diameter of 0.20 mm on the left renal artery, leaving the right kidney undisturbed. The rats developed hypertension within 3–4 weeks as has been shown before[8,9]. Blood pressure measurements were carried out with a tail sphygmographic method[8]. All rats were kept on a 14 h light, 10 h dark schedule, with lights on from 05.00 h–19.00 h and had access to water and rat chow ad lib.

Noradrenaline concentrations and α-MPT-induced noradrenaline disappearance in brain regions

On the last day of the experiment, 4 groups of rats were decapitated; one group of hypertensive rats and a group of control rats were killed without prior treatment with α-MPT. Two hours after the enzyme tyrosine hydroxylase (TH) had been

inhibited by a single dose (300 mg/kg i.p.) of DL-α-methyl-para-tyrosine-methylester (α-MPT, AB Biotec, Göteborg), another group of hypertensive and of control rats were decapitated. After decapitation the brains were rapidly taken out and cut into 300 μm serial sections in a cryostat at —10 °C. The following 8 nuclei were punched out from the frozen sections according to the method of Palkovits[12]: the nucleus interstitialis striae terminalis (NIST), the nucleus periventricularis (NPE), the nucleus paraventricularis (NPV), the nucleus hypothalamicus anterior (NHA), the A_1-catecholaminergic cell groups (lateral reticular formation) and 3 parts of the nucleus tractus solitarii, viz. the nucleus commissuralis (NCO), the A_2-catecholaminergic cell groups (caudal to the obex) and the nucleus tractus solitarii proper (rostral to the obex) (for details see ref. 22). Tissue pellets from two rats were pooled. The noradrenaline content was assayed as previously described[21] and expressed as pg/μg protein.

The α-MPT-induced noradrenaline disappearance was expressed as the ratio of the noradrenaline content 2 h after α-MPT and the steady state content × 100%. Data are given as means ± S.E.M. Tests of significance were performed using Students *t*-test. *P* values of less than 0.05 were regarded as indicating a significant difference.

RESULTS

Animals

The data concerning the blood pressure, heart rate and body weight are summarized in Table I. An increase in blood pressure was detected 3 days after application of the clip, the mean blood pressure being 131 mm Hg vs 119 mm Hg for the controls. After 3.5 weeks, a value of 204 mm Hg was measured for the hypertensive rats. The heart rate of the hypertensive rats was also elevated at 3 days and at 3.5 weeks. At 3.5 weeks after the application of the artery clip the body weight of the hypertensive rats was significantly less than that of the sham operated rats.

TABLE I

Body weight, blood pressure and heart rate of sham operated and two-kidney Goldblatt hypertensive rats 3 days and 3.5 weeks after the induction of hypertension

Values are means ± S.E.M. (n = 24–28)

	3 Days		*3.5 Weeks*	
	Sham operated	*Hypertensive*	*Sham operated*	*Hypertensive*
Body weight (g)	133 ± 4	132 ± 2	190 ± 10	168 ± 8*
Blood pressure (mm Hg)	119 ± 0.4	131 ± 2*	123 ± 0.5	205 ± 3***
Heart rate (b.p.m.)	320 ± 2	360 ± 4**	335 ± 3	428 ± 4***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for differences between sham operated and hypertensive rats.

Noradrenaline concentrations and α-MPT-induced noradrenaline disappearance in brain regions

Measurable amounts of noradrenaline were detected in all nuclei. At 3 days after the operation, the noradrenaline concentration of the A_1-region of renal hypertensive rats was significantly higher than that of control rats (Table II). The α-MPT-induced noradrenaline disappearance was decreased in two hypothalamic nuclei, viz. the NIST and the NPV of these rats (Table II). At this early stage of hypertension the disappearance of noradrenaline was found to be increased in two nuclei of the medulla oblongata, viz. the A_1-region and the NCO (Table II). At 3.5 weeks after the induction of hypertension no significant changes were observed in the noradrenaline content or in the α-MPT-induced noradrenaline disappearance in the various nuclei (Table II).

TABLE II

Steady-state noradrenaline concentration and the noradrenaline concentration 2 h after α-MPT of discrete regions of the brain of sham-operated (N) and two-kidney Goldblatt hypertensive rats (H) 3 days and 3.5 weeks after the induction of hypertension

For details see text. Values are means ± S.E.M. (n = 6–7). Abbreviations used: NIST, nucleus interstitialis striae terminalis; NPE, nucleus periventricularis; NPV, nucleus paraventricularis; NHA, nucleus hypothalamus anterior; A_1, A_1-catecholaminergic cell group of the lateral reticular nucleus; NCO, nucleus commisuralis; A_2, A_2-catecholaminergic cell group; NTS, nucleus tractus solitarii proper.

Brain region	*Noradrenaline concentration*			
	3 Days		*3.5 Weeks*	
	0 h Conc. (pg/μg prot.)	*Conc. 2 h after α-MPT (% of 0 h conc.)*	*0 h Conc. (pg/μg prot.)*	*Conc. 2 h after α-MPT (% of 0 h conc.)*
NIST	N 40.70 ± 6.74	41 ± 7	37.15 ± 7.19	41 ± 5
	H 29.90 ± 2.80	89 ± 7*	48.15 ± 5.67	51 ± 11
NPE	N 34.51 ± 3.48	64 ± 6	31.43 ± 2.10	58 ± 6
	H 39.59 ± 2.58	72 ± 3	32.26 ± 1.81	50 ± 3
NPV	N 39.07 ± 6.28	40 ± 14	35.49 ± 3.85	37 ± 3
	H 32.47 ± 5.68	95 ± 8*	39.01 ± 5.64	35 ± 5
NHA	N 12.86 ± 0.85	51 ± 9	11.71 ± 0.97	48 ± 7
	H 13.65 ± 1.36	69 ± 7	12.09 ± 1.02	45 ± 7
A_1	N 16.28 ± 1.08	61 ± 8	14.79 ± 1.07	63 ± 4
	H 25.55 ± 2.99***	33 ± 3*	12.90 ± 1.09	55 ± 7
NCO	N 22.95 ± 2.58	72 ± 6	20.92 ± 1.51	69 ± 6
	H 26.14 ± 5.43	55 ± 4**	20.70 ± 1.27	60 ± 5
A_2	N 32.39 ± 0.11	64 ± 6	29.48 ± 3.48	60 ± 7
	H 31.59 ± 1.24	50 ± 3	29.74 ± 2.99	62 ± 13
NTS	N 14.97 ± 1.21	61 ± 6	13.67 ± 3.49	65 ± 6
	H 11.91 ± 1.56	57 ± 10	14.49 ± 1.90	44 ± 13

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

DISCUSSION

In addition to peripheral factors, changes in the central nervous system may determine the development of hypertension. The activity within the baroreceptor neurons is enhanced as a consequence of the gradual rise in blood pressure. These neurons have their first synapse within the mediocaudal part of the NTS. The NTS is interconnected with several nuclei in the medulla oblongata as well as with nuclei in the hypothalamus[3,13]. Following small lesions in the NTS, nerve terminal degeneration was observed within the intact parts of the NTS (viz. the A_2-region and the NCO) and in the nucleus reticularis lateralis (containing the A_1-region) and some other pons-medulla nuclei[13]. In the early stage of two-kidney Goldblatt hypertension (3 days post-operative) the α-MPT-induced noradrenaline disappearance was reduced in two nuclei of the anterior hypothalamus, viz. the NIST and the NPV. At the same time noradrenaline disappearance was enhanced in the A_1-region and the NCO of the medulla oblongata. In a later stage of hypertension, 3.5 weeks after the operation, the noradrenergic activity in these nuclei had returned to control levels. In one-kidney Goldblatt hypertensive rats Petty and Reid found a transient reduction in the concentration of noradrenaline in various nuclei in the medulla oblongata and in the hypothalamus in the NHA, NPV, posterior hypothalamic nucleus, the NTS-complex and the lateral reticular nucleus[15]. In a subsequent paper, Petty and Reid[16] reported that the reduction in noradrenaline concentration in the hypothalamic nuclei was accompanied by a decreased TH activity; in the NTS-complex and the lateral reticular nucleus a non-significant elevation in TH activity was found. However, pathophysiological differences appear to exist between the one-kidney model used by Petty and Reid and the two-kidney model used in the present study (see Introduction).

As mentioned above, a rise of blood pressure will be accompanied by an increase of activity in the baroreceptor pathways. It has recently been shown that connections exist between the A_2-region and the hypothalamus and vice versa[13]. The enhanced baroreceptor activity, in its turn might therefore lead to a changed activity of noradrenaline neurons which project to the hypothalamus and are involved in the regulation of arterial blood pressure. In favor of the involvement of noradrenergic receptors are the findings of Struyker Boudier et al.[19,20], who observed that microinjections of noradrenaline or adrenaline in this area resulted in a decrease in blood pressure, and of Hilton[7] who observed that electrical stimulation of the anterior hypothalamus caused a fall in arterial pressure in dogs. These observations indicate that the arterial blood pressure can be reduced by an activation of noradrenergic receptors in the hypothalamus.

The present results, however, indicate that in the early stage of renovascular hypertension, the activity of noradrenaline containing neurons to the anterior hypothalamus is decreased. It might be that, (1) either the noradrenaline-containing neurons terminating in the nuclei in which noradrenergic activity was reduced are not integrated in such a depressor system or, (2) that the reduced activity in that system and the supposedly corresponding decrease in the activation of noradrenergic receptors in the anterior hypothalamus contribute to or facilitate the rise in arterial pressure.

Recently we have reported that in the initial phase of the development of spontaneous hypertension the α-MPT-induced disappearance of noradrenaline was decreased in nuclei in the anterior hypothalamus[23]. A transient increase in sympathetic nerve activity has been shown in this form of hypertension[11]. We suggested that the reduction of the noradrenergic activity in nuclei in the anterior hypothalamus in young SH-rats could be related to the augmented sympathetic outflow. It is unknown whether a transient rise in sympathetic outflow during the development of hypertension also occurs in renal hypertension. While there are similarities in the changes in the activity in the anterior hypothalamus in spontaneous and in renal hypertension, the peripheral mechanisms related to the elevated blood pressure seem to be different. In the development of two-kidney Goldblatt hypertension, the increased activity in the renin–angiotensin system is considered to play an important role[2,4,6,9]. Data concerning the activity of the renin–angiotensin system during the development of spontaneous hypertension are conflicting. Several groups of investigators have found plasma renin activity (which reflects the activity of the renin–angiotensin system) in SH-rats to be either decreased, increased or unchanged (see ref. 18 and references therein). It is worth mentioning that electrolytic lesions around the anteroventral third ventricle, known as a site of central angiotensin dipsogenic and pressor mechanisms, inhibited the development of different forms of hypertension but not of spontaneous hypertension[1].

Our data indicate that there is a change in activity during the resetting phase of the early stage of hypertension in noradrenaline neurons in nuclei of both anterior hypothalamus and the medulla oblongata of two-kidney Goldblatt rats. However, it is not clear whether this changed activity results from the developing hypertension or contributes to its development.

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