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Regional Brain Catecholamine Levels and the Development of Hypertension in the Spontaneously Hypertensive Rat: the Effect of 6-Hydroxydopamine

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To investigate the role of central catecholaminergic pathways in the development of hypertension in the spontaneously hypertensive rat (SHR) the effects of intracerebroventricular (i.c.v.) injections of 6-hydroxydopamine (6-OHDA) were compared with those of local injections near the main ascending noradrenergic pathways. The parameters studied were systolic blood pressure, heart rate and regional catecholamine concentrations in micropunched brain areas. I.c.v. treatment with 6-OHDA (three 200 μg injections) of young SHR attenuated the development of hypertension and caused widespread depletion of noradrenaline and to a lesser extent of dopamine and adrenaline. 6-OHDA-induced lesions of the dorsal and ventral noradrenergic bundles did not affect the rise in blood pressure but induced a depletion of forebrain noradrenaline comparable to that after the i.c.v. treatment. Dopamine and adrenaline levels were, however, not substantially affected. These results suggest that forebrain noradrenergic innervation may not be of major importance for the development of hypertension in the SHR.

INTRODUCTION

A considerable number of studies suggest involvement of brain catecholaminergic systems in the initiation of the rise in blood pressure in young spontaneously hypertensive rats (SHR). Already in 1972, Haeusler and co-workers showed that intracerebroventricular (i.c.v.) administration of 6-hydroxydopamine (6-OHDA) to 6–7 weeks old SHR prevents the development of hypertension in these animals, while the same treatment in adult SHR did not result in permanent changes in blood pressure[10,11]. The neurotoxin 6-OHDA is known to specifically deplete catecholamine stores from nerve terminals and to lead to retrograde degeneration of catecholamine neuron systems[13,14]. Thus, SHR treated with high doses of i.c.v. administered 6-OHDA showed significant depletion of noradrenaline and dopamine in brain and spinal cord[5,15]. Intraspinal injections of 6-OHDA, resulting in depletion in the spinal cord only, did not, however, interfere with the rise in blood pressure[15], suggesting a specific brain site for the effects observed after i.c.v. 6-OHDA injections.

There is also more indirect evidence for the involvement of brain catecholaminergic systems in the development of hypertension in the SHR. Several authors have reported age-dependent changes in the levels and/or turnover of noradrenaline, dopamine and also adrenaline in a variety of brain structures[6,9,12,17–19,23,24,28,29,34,36–38]. The results of these studies are often conflicting and do not fit together to form a clear concept of the role of central catecholamines in the initiation or the maintenance of high blood pressure in the SHR. In a number of these studies, however, changes in catecholamine levels were found in the hypothalamus of young 'prehypertensive' SHR as compared to age-matched Wistar-Kyoto control rats. Together with the finding that hy-

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pothalamic catecholamines are implicated in the regulation of basal blood pressure[1-3,6], these data point towards a possible role of forebrain catecholaminergic innervation in the development of high blood pressure in these animals. The present study was undertaken to further investigate this possibility, with emphasis on the question which of the catecholamine systems is of major importance.

Forebrain noradrenergic innervation originates in medullary cell groups and is carried through the periventricular bundle and the dorsal and ventral bundles[20,26,27,32]. 6-OHDA-induced lesions of these ascending neuronal bundles lead to depletion of noradrenaline in forebrain structures[14,32], while leaving dopaminergic innervation intact. Adrenaline neurons also ascend from medullary nuclei, but take a route distinct from the noradrenergic bundles[26] and will probably not be damaged by the treatment. Comparison of blood pressure effects and regional depletion values after these specific lesions and after i.c.v. 6-OHDA treatment according to the protocol of Haeusler and colleagues (see above), which presumably affects all 3 catecholamines, were anticipated to give better understanding of the role of brain catecholamines in general and forebrain noradrenergic innervation in particular in the process of the rise in blood pressure in the SHR.

MATERIALS AND METHODS

Male SHR derived from a colony of SHR-NIH cpb were used. The animals were weaned at the age of 4 weeks and kept under a constant light–dark regime and had food and water available ad libitum.

For i.c.v. injections, the animals were operated shortly after weaning under Hypnorm anesthesia. As 6-OHDA appears to have asymmetrical effects after unilateral ventricular administration[8], the rats were provided with bilaterally placed polyethylene cannulas in the lateral cerebral ventricles. After a recovery period of 7 days, the animals received either 6-OHDA, dissolved as 200 μg base per 10 μl physiological saline with ascorbic acid (0.2 mg/ml), or only the same volume of vehicle. Solutions were freshly prepared before the injections and were kept on ice. Perfusion-time per injection was about 2 min. Injections were tolerated well. The first 10 μl injection was administered in the left ventricle. 48 h later the second 10 μl was injected through the right ventricular cannula. Again 48 h later the third dose was given as two bilateral 5 μl injections. Thus, the 6-OHDA-treated rats received a total dose of 600 μg base. This protocol is an adaptation of the original injection-schedule of Haeusler and colleagues[10,11]. A lower dose was chosen (200 μg instead of 250 μg) to avoid severe toxicity. After the third injection the rats were allowed a recovery period of 3 days after which training procedures were started for indirect blood pressure determination.

Measurement of systolic blood pressure and heart rate were carried out on conscious animals with a tail-plethysmographic method as reported earlier[16]. In general, the development of hypertension was followed at least 3 weeks, after which, in case of relatively constant levels of blood pressure, the rats were decapitated and their brain taken out for catecholamine assay. The procedure consisted of rapidly freezing the brains on dry ice, cutting serial sections of 300 μm on a cryostat at —10 °C, and punching out a selected group of individual brain regions with hollow needles[25]. The pellets from individual rats were homogenized in 70 μl 0.1 N $HClO_4$. Protein content was assayed in diluted samples of the homogenate according to the method of Lowry[22]. Noradrenaline, dopamine and adrenaline were assayed in 20 μl samples of the homogenate with a sensitive radio-enzymatic assay according to the method of Van der Gugten[33]. Slight modifications were incorporated in this method in that blanks were prepared without adding magnesium and internal standards of the catecholamines were assayed separately. Cross-over was corrected for. Cross-over values in the assays used for the present experiments were: dopamine into noradrenaline 3.8%, dopamine into adrenaline 4.1%, adrenaline into dopamine 3.8%, adrenaline into noradrenaline 1.8%, noradrenaline into dopamine 3.4%, noradrenaline into adrenaline 1.2%. Catecholamine concentrations are expressed as pg per μg protein or as percentage of control values.

In the second experiment 5-week-old male SHR were injected bilaterally under ether anaesthesia directly into the midbrain. Stereotactic coordinates with the toothbar at zero and the bregma as zero were: posterior 5.1 mm, lateral 0.6 mm and ventral 6.3 mm for the first injection; posterior 4.6 mm, lateral 1.2 mm and ventral 7.8 mm for the second injec-

tion. With each injection 10 μg of 6-OHDA was administered in 1 μl physiological saline with ascorbic acid (0.2 mg/ml) using a Hamilton microsyringe via a guide cannula. Thus, 6-OHDA-treated rats received a total dose of 40 μg base. Control animals received vehicle injections only. After recovery of the animals the same procedure was followed as in the first experiment, except that after decapitation the hindpart of the brain was fixed in formalin and processed for routine histology to determine the location of the cannula-tips. Fig. 1 shows a composite drawing of the position of the injections in the combined 6-OHDA-injected and vehicle-injected group. Rats displaying differences with these positions were discarded from the biochemical assay. No tissue necrosis or local alterations were found around the tip of the cannula.

Data are expressed as mean ± standard error of the mean (S.E.M.). For statistical comparison Student's *t*-test was used. When $P < 0.05$ (two-tailed) differences between the groups were considered statistically significant.

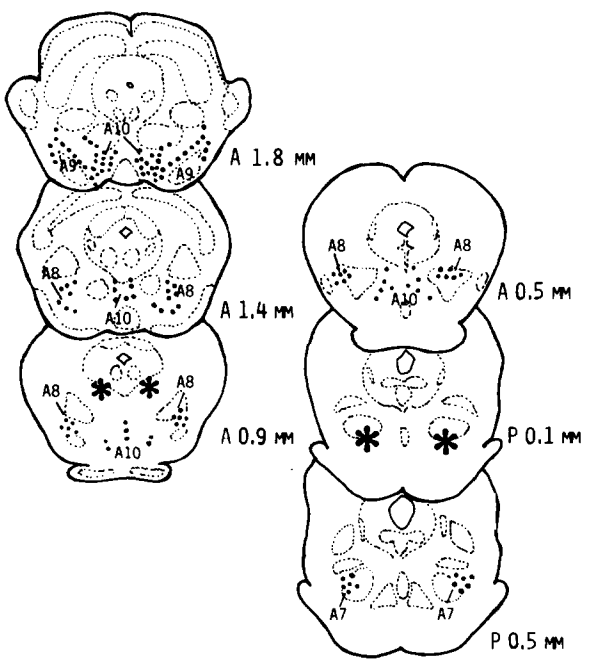

Fig. 1. Schematic drawing of the location of the cannula-tips (asterisks) as used for micro-injection of 6-OHDA or vehicle. Drawing adapted from the atlas of Palkovits and Jacobowitz[27]. The anterior/posterior zero refers to the interaural line. Regions of origin of catecholamine fiber systems (A7, A8, A9 and A10 region) are indicated by black dots.

RESULTS

In i.c.v. 6-OHDA-treated rats, especially the first injection induced a transient period of hypomotility and after a few hours a hyperreactivity to tactile and environmental stimuli. During the one-week training procedures for blood pressure determination this hyperreactivity slowly disappeared. For the remaining experimental period i.c.v. 6-OHDA-treated rats appeared to have decreased locomotor activity as compared to their vehicle-injected controls. Midbrain micro-injected animals, either with vehicle or with 6-OHDA, did not show any changes in gross behaviour after recovery from anaesthesia.

Body weight changes also followed different patterns in the two experiments. I.c.v. 6-OHDA-injected rats, again mainly after the first injection, showed a slight attenuation of body weight increase and in some animals even a transient decrease. Already before the third injection body weight increased again in most of the animals and the curve of the 6-OHDA-treated rats thereafter paralleled that of controls. At the end of the experiment no differences were observed in body weight between the experimental groups. In the second experiment, no changes in body weight gain were seen after local midbrain injections (data not shown).

The development of hypertension in the i.c.v. 6-OHDA-treated SHR was markedly attenuated, as shown by significantly lower systolic blood pressure values during the whole measurement-period (Fig. 2a). Vehicle-injected rats had systolic blood pressures up to 210 mm Hg, while these values in the 6-OHDA-injected SHR generally did not exceed 170 mm Hg. SHR injected locally with 6-OHDA near the noradrenergic bundles developed hypertension similarly to vehicle-injected rats. Systolic blood pressure values between these groups were not significantly different during the entire period of measurement (Fig. 2b).

Rats injected i.c.v. with 6-OHDA displayed significantly lower heart rates than vehicle-injected controls. The average heart rate in vehicle-injected rats was 450 beats per minute, while that of 6-OHDA-injected rats was only about 350 beats per minute (Fig. 3a). In the locally injected groups no consistent difference was found between the vehicle-injected rats and the 6-OHDA-treated group (Fig. 3b). In

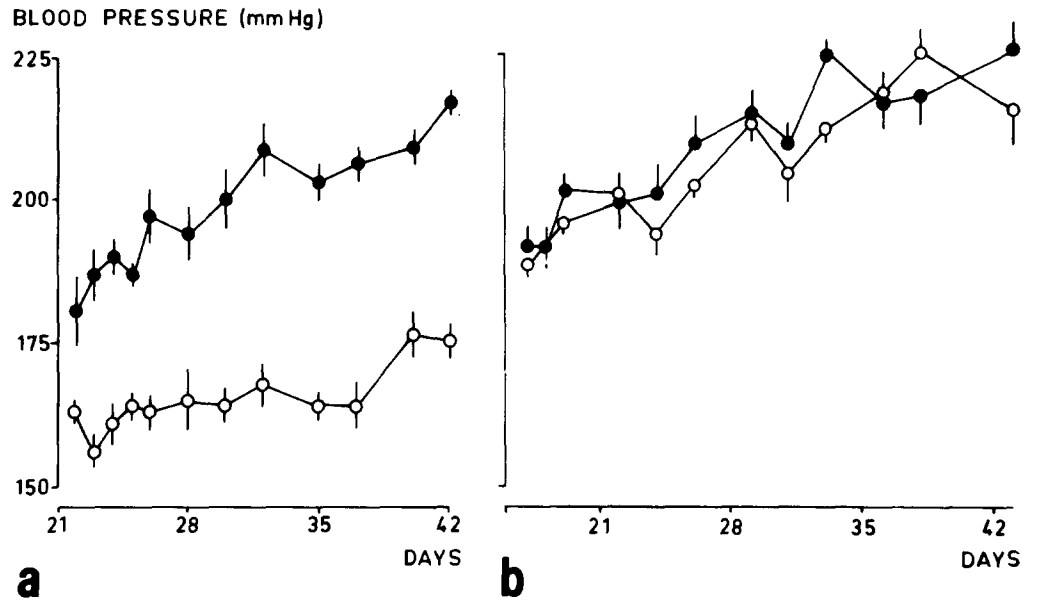

Fig. 2. Systolic blood pressure of SHR after i.c.v. injections of 6-OHDA or vehicle (a) or after local midbrain micro-injection of 6-OHDA or vehicle (b). In both cases filled circles indicate values for vehicle-injected animals and open circles indicate values for 6-OHDA-injected rats. The number of days on the abcissa refers to the time after the first injection (day zero). For both i.c.v. treated groups n = 7; for the locally vehicle-injected group n = 9 and for the locally 6-OHDA-injected group n = 12. After i.c.v. 6-OHDA injections on every day of measurement a significant difference ($P < 0.05$) was found between the groups (a). After local micro-injections on none of the days of measurement a significant difference was found between the groups (b).

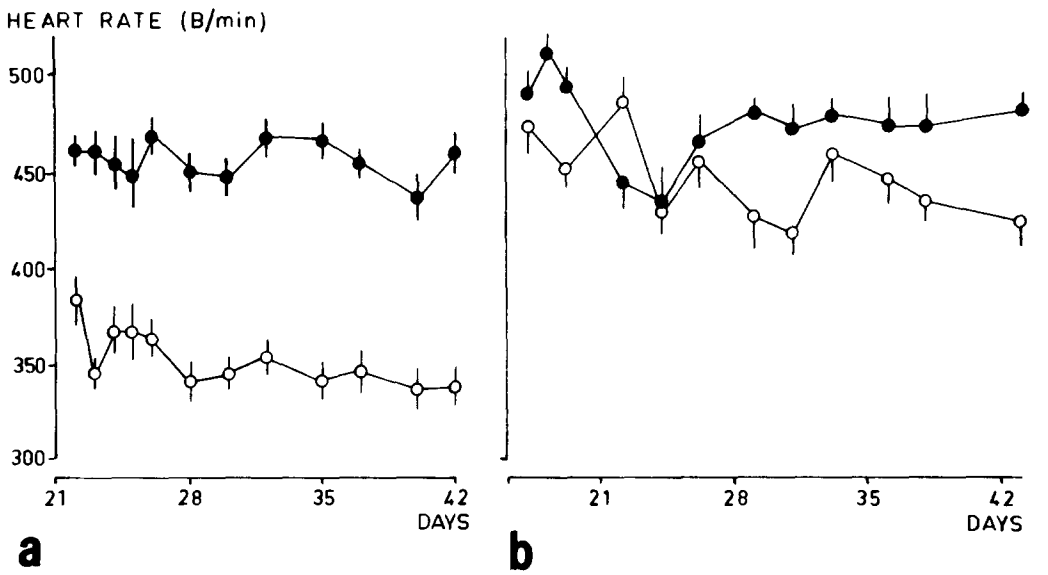

Fig. 3. Heart rate of SHR after i.c.v. injections of 6-OHDA or vehicle (a) or after local midbrain micro-injections of 6-OHDA or vehicle (b). In both cases filled circles indicate values for vehicle-injected animals and open circles indicate values for 6-OHDA-injected rats. The number of days on the abcissa refers to the time after the first injection (day zero). For both i.c.v. treated groups n = 7; for the locally vehicle-injected group n = 9 and for the locally 6-OHDA-injected group n = 12. After i.c.v. 6-OHDA treatment on every day of measurement a significant difference ($P < 0.05$) was found between the groups (a). After local 6-OHDA micro-injections on days 29, 31 and 42 only a significant difference ($P < 0.05$) was found between the groups (b).

this experiment controls displayed a heart rate of 470 beats per minute, while 6-OHDA-injected SHR had a heart rate of around 440 beats per minute.

Biochemical results indicate differential effects of both treatments on catecholamine concentration in the selected micropunched brain areas. The catecholamine concentration of various brain regions of adult SHR which had been injected i.c.v. with 6-OHDA or vehicle at the age of 5 weeks are shown in Table I. Noradrenaline was significantly depleted in all areas investigated except the rostral part of the nucleus tractus solitarii (NTS) and the locus coeruleus. A tendency towards a difference, however, existed in the locus coeruleus, although the decrease did not reach statistical significance, due to large variation in the control group. Noradrenaline levels were below the limit of detection in the nucleus accumbens and the nucleus caudatus. Dopamine and adrenaline were affected less and in fewer brain areas than noradrenaline. Dopamine was significantly depleted in frontal cortex, nucleus accumbens, nucleus caudatus, lateral septal nucleus, nucleus interstitialis striae terminalis (NIST), central amygdaloid nucleus, nucleus hypothalamus anterior and nucleus dorsomedialis. Adrenaline was only detectable in hypothalamic and medullary regions, as well as in the zona incerta and the central amygdaloid nucleus. A significant depletion was found only in the paraventricular, periventricular, arcuate and dorsomedial nuclei.

For better comparison between the effects of i.c.v. injections of 6-OHDA and midbrain micro-injections, data in Fig. 4 are expressed as percentage of their respective control-values. The absolute concentration found in both experiments did not differ from those reported previously[34,35] (see also Table I). 6-OHDA-induced lesions of the ascending noradrenergic bundles induced a strong depletion of noradrenaline in the forebrain nuclei studied. Fig. 4 shows that noradrenaline values in the regions investigated in both experiments are decreased to a comparable extent. Local midbrain injection of 6-OHDA did not deplete dopamine except for a small but significant reduction in the zona incerta. Dopamine concentration in the frontal cortex and the nucleus periventricularis, however, were markedly increased after midbrain injections of 6-OHDA as compared to vehicle-injected control-values. In contrast to i.c.v. adminis-

TABLE I

Catecholamine concentration of individual micropunched brain areas of SHR after i.c.v. injections of 6-OHDA or vehicle

Catecholamines are expressed as pg/μg protein ± S.E.M. For both groups n = 7.

	Noradrenaline		*Dopamine*		*Adrenaline*	
	Vehicle	*6-OHDA*	*Vehicle*	*6-OHDA*	*Vehicle*	*6-OHDA*
Brain region						
Nucleus accumbens			69.7 ± 3.6	47.0 ± 4.2**		
Frontal cortex	4.3 ± 0.3	0.2 ± 0.06**	2.6 ± 0.3	1.5 ± 0.2*		
Nucleus caudatus			73.6 ± 3.9	46.8 ± 5.2*		
Lateral septal nucleus	15.8 ± 1.1	1.8 ± 0.4**	14.3 ± 1.3	2.1 ± 0.3**		
Nucl. interst. striae termin.	43.6 ± 6.6	15.4 ± 3.9*	15.8 ± 2.5	6.6 ± 1.4*		
Paraventricular nucleus	46.2 ± 7.9	20.1 ± 3.7*	5.5 ± 0.9	5.0 ± 1.0	1.8 ± 0.2	0.8 ± 0.1*
Periventricular nucleus	50.8 ± 1.0	7.7 ± 0.7**	8.3 ± 0.3	7.4 ± 0.8	1.4 ± 0.1	0.5 ± 0.2**
Anterior hypothalamic nucleus	24.6 ± 3.0	8.7 ± 1.0**	3.0 ± 0.2	2.3 ± 0.1*	0.6 ± 0.1	0.7 ± 0.1
Central amygdaloid nucleus	12.3 ± 1.7	3.7 ± 0.8**	14.3 ± 2.1	3.7 ± 0.6**	0.6 ± 0.2	0.6 ± 0.1
Arcuate nucleus	26.4 ± 1.8	6.3 ± 1.2**	5.3 ± 0.7	6.1 ± 1.0	1.2 ± 0.07	0.5 ± 0.1**
Median eminence	16.0 ± 3.1	4.9 ± 1.5*	36.1 ± 2.3	28.1 ± 7.2		
Dorsomedial nucleus	56.0 ± 6.0	13.1 ± 0.8**	5.8 ± 0.7	3.7 ± 0.2*	4.9 ± 0.06	1.5 ± 0.2**
Gyrus dentatus	18.4 ± 1.9	1.8 ± 0.4**	2.5 ± 0.6	3.6 ± 1.1		
Zona incerta	23.6 ± 2.0	7.9 ± 2.1**	4.7 ± 0.6	3.4 ± 0.4	2.5 ± 0.5	1.7 ± 0.2
A1 region	24.6 ± 3.4	9.3 ± 0.4*	3.6 ± 0.5	3.5 ± 0.2	2.3 ± 0.3	2.2 ± 0.07
A2 region	90.4 ± 5.4	42.3 ± 2.3**	11.7 ± 1.4	8.4 ± 1.2	4.9 ± 0.9	4.4 ± 0.4
Rostral NTS	30.7 ± 4.5	22.8 ± 2.6	4.4 ± 0.6	5.6 ± 0.3	3.5 ± 0.4	3.8 ± 0.4
Locus coeruleus (A6 region)	43.5 ± 13.7	18.1 ± 2.6	9.7 ± 2.0	5.7 ± 0.7	4.2 ± 1.2	3.1 ± 0.4

* $P < 0.05$; ** $P < 0.001$.

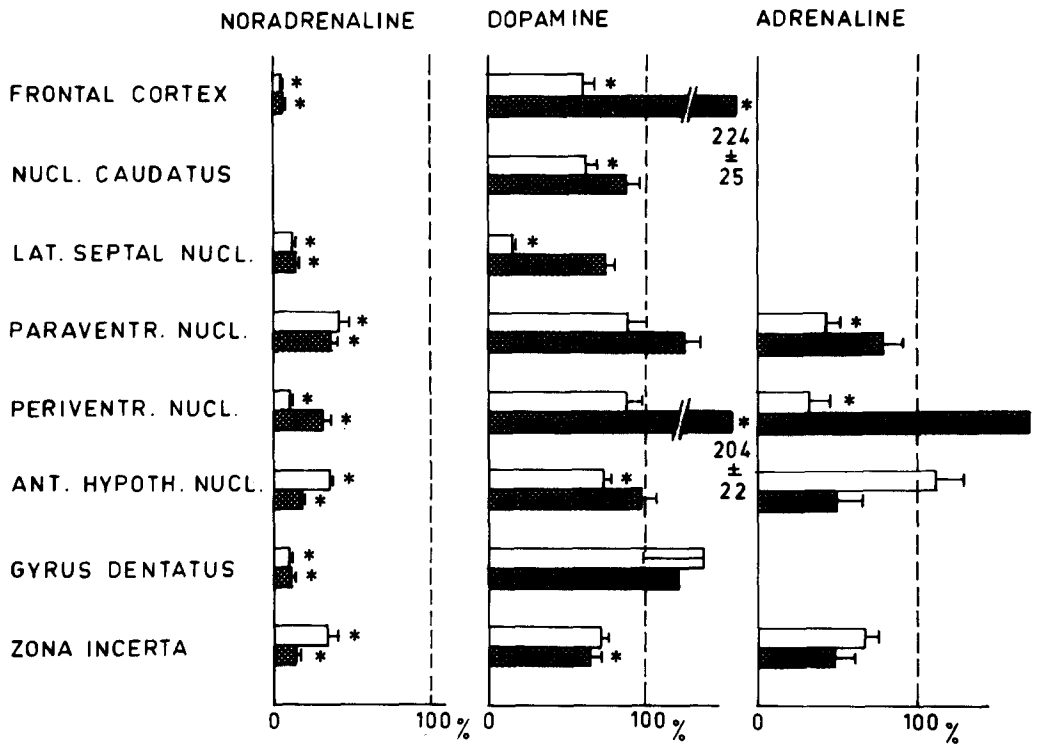

Fig. 4. Brain catecholamine concentrations of SHR after i.c.v. 6-OHDA-treatment (open bars) or after local midbrain micro-injections of 6-OHDA (stippled bars) as percentage of their respective control values found in vehicle-injected rats. *indicates statistically significant ($P < 0.05$) difference between 6-OHDA-treated and vehicle-treated groups of either treatment.

tration local injections of 6-OHDA near the noradrenergic bundles did not induce significant effects on adrenaline concentration in the regions studied, although tendencies towards a difference were sometimes observed.

DISCUSSION

In this study the effects of i.c.v. injections of high doses of 6-OHDA on the development of hypertension in the SHR were compared with those of midbrain micro-injection of the neurotoxin near the dorsal and ventral noradrenergic bundles. The rise in blood pressure was markedly attenuated in i.c.v. 6-OHDA-treated rats as compared to vehicle-injected controls, while local micro-injections of 6-OHDA in the midbrain did not induce changes in the development of hypertension. Both treatments had comparable effects on forebrain noradrenergic levels but not on dopamine and adrenaline concentrations.

The effect of i.c.v. injections of 6-OHDA on the development of hypertension in the SHR as found in the present study corroborates the results of the original study of Haeusler and colleagues[10] as well as those of later studies[5,15,21]. Heart rate was also significantly lower in i.c.v. 6-OHDA-treated SHR in our experiments, which has been reported earlier too[5,15].

For a detailed examination on the regional effects of 6-OHDA-treatment on the levels of noradrenaline, dopamine and adrenaline in SHR, in this study selected individual catecholaminergic terminal areas[20,32] were punched from brain slices of both i.c.v. 6-OHDA-injected and vehicle-injected SHR.

The most pronounced effect of i.c.v. 6-OHDA-treatment was found on the noradrenergic systems. Marked depletions occurred in the frontal cortex and gyrus dentatus, areas which are known to receive input from the locus coeruleus via the dorsal noradrenergic bundle. However, depletion of noradrenaline in regions of origin of the ventral bundle, the A1 and A2, as well in the hypothalamic areas which receive mixed noradrenergic innervation indicates that also the ventral bundle system is affected.

In the paraventricular nucleus, periventricular nu-

cleus and anterior hypothalamic nucleus of young SHR a decreased noradrenergic turnover as compared to Wistar-Kyoto controls has been found[37]. In the present experiment i.c.v. 6-OHDA administration induced a strong depletion of noradrenaline in these nuclei. Lesions of the central amygdala were recently shown to attenuate the rise in blood pressure in SHR[7]. Noradrenaline concentrations in the central amygdaloid nucleus as well as in the nucleus of the main ascending route to the amygdala, the NIST, were reduced after 6-OHDA-treatment. Noradrenergic depletion in the arcuate nucleus and the median eminence may have implications for the regulation of the endocrine system in the treated SHR.

Depletion values of dopamine after i.c.v. 6-OHDA show that areas innervated both by the A9 and A10 are affected. However, there is not a general depletion of dopamine. The largest effect was seen in the lateral septal nucleus, which might be explained by the relatively short distance of this area to the lateral ventricle cannulas where concentrations of 6-OHDA could reach high values. The depletion of dopamine in the central amygdaloid nucleus and the NIST is again of interest in view of the lesion studies suggesting a role of the amygdala in the development of spontaneous hypertension[7]. Significant depletion of dopamine in the dorsomedial nucleus of the hypothalamus might implicate that the incerto-hypothalamic dopamine system is also affected by the i.c.v. 6-OHDA-treatment in the present experiments.

Adrenaline depletion was found in some, but not all hypothalamic nuclei and in none of the regions of origin in the medulla. Forebrain adrenaline innervation has been implicated in the regulation of blood pressure[30] and adrenaline levels were found to be changed in hypothalamic nuclei of SHR of various ages[35,36]. Depletion of adrenaline might, therefore, also contribute to the 6-OHDA-induced attenuation of the development of hypertension.

Since i.c.v. treatment was found to affect noradrenaline innervation most markedly, and the ascending noradrenergic bundles are clearly defined[27,32], 6-OHDA-induced lesions of the dorsal and ventral bundles were made in SHR of the same age as in the first experiment. A representative selection of forebrain nuclei from the first experiment was used because the bundles lesioned project almost entirely to the forebrain and after lesioning depletion patterns were expected to be general. Comparison of the effects of both treatments reveals an almost identical depletion of noradrenaline, whereas the pattern for dopamine and adrenaline is different. Since the site of the injections is located caudally to the A9 and A10 dopamine cell groups (Fig. 1), it is not surprising that no general depletion of dopamine occurred. Effects on the dopaminergic system after local micro-injections in the noradrenergic bundles were found only in the frontal cortex, periventricular nucleus of the hypothalamus and in the zona incerta. An increase in dopamine concentration after noradrenaline depletion, as found in frontal cortex and periventricular nucleus, has been reported previously for the frontal cortex[4,31]. The extent of the increase was, however, only up to 40% in these studies, while the present experiments reveal a much larger effect. This quantitative difference may be explained by strain differences. SHR have been found to have increased dopaminergic receptor binding[17], a decreased dopamine neuronal uptake[23] and higher dopamine concentration[35] in the frontal cortex as compared to Wistar-Kyoto controls. Also for the observed increased dopaminergic concentration in the periventricular nucleus, as well as for the decrease in dopamine content in the zona incerta, an interaction between noradrenergic and dopaminergic systems may be postulated. Damage to the dopamine-system itself after i.c.v. 6-OHDA could block the effect of noradrenaline depletion. The decrease in zona incerta dopamine concentration is unlikely to be caused by direct effects of the 6-OHDA micro-injections on the most nearby dopamine cellgroup, the A8, since in pilot-studies it was found that this effect was also present in animals with lesions in the dorsal bundle only (data not shown).

No significant effects were found on adrenaline concentrations after 6-OHDA micro-injection, although in some nuclei tendencies were observed.

Thus, micro-injections of 6-OHDA as described here seem to induce a specific depletion of forebrain noradrenergic innervation. Hindbrain catecholamine concentrations were not investigated in this study. 6-OHDA lesions of the ascending noradrenergic bundles, however, failed to affect the development of hypertension in the SHR. This could suggest that forebrain noradrenergic innervation does not play a major role in the development of hyperten-

sion in the SHR. The present results do not, however, give information concerning the time-course of the destruction of the catecholamine systems. Since i.c.v. injections of 6-OHDA will affect terminals first[13,14] and the lesions in the second experiment were directed towards axonal bundles, one should consider the possibility that the depletion values we observed at the termination of our experiments developed through different mechanisms. Measurement of catecholamine turnover or metabolites could give information to solve these problems.

Erinoff and co-workers have also found a normal development of high blood pressure in SHR with midbrain noradrenergic bundle lesions[5]. These authors used mechanical lesions rather than chemical ones and the site of the lesion was restricted to the dorsal bundle mainly. Our present results extend these findings since a more specific depletion was achieved through the use of 6-OHDA. In addition, catecholamines were assayed in micropunched brain regions and the ventral bundle was lesioned in all animals.

In conclusion, the present results show that i.c.v. injections of 6-OHDA induce an attenuation of the development of hypertension in SHR and a marked depletion of brain noradrenaline. Destruction of forebrain noradrenergic innervation itself, however, has no effects on the rise in blood pressure in treated SHR. Further investigation is needed to study the role of medullary noradrenergic innervation, as well as brain dopamine and adrenaline systems, in the development of hypertension in the SHR.

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