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Central 6-OHDA Affects Both Open-Field Exploratory Behaviour and the Development of Hypertension in SHR

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VAN DEN BUUSE, M., H. D. VELDHUIS, S. DE BOER, D. H. G. VERSTEEG AND W. DE JONG. *Central 6-OHDA affects both open-field exploratory behaviour and the development of hypertension in SHR.* PHARMACOL BIOCHEM BEHAV **24**(1) 15–21, 1986.—To investigate the possible relation between changes in behaviour and the development of hypertension in the spontaneously hypertensive rat (SHR), the effect of intracerebroventricular (ICV) administration of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) on blood pressure and spontaneous behaviour was studied. In addition to an attenuation of the rise in blood pressure in SHR, 6-OHDA induced a marked decrease in rearing activity in the open field towards levels found in WKY. Other parameters were either not changed (stereotyped sniffing) or influenced in a comparable way in SHR and WKY (increase in locomotion). These results suggest that ICV 6-OHDA may simultaneously affect the development of hypertension and certain components of the changed behaviour of SHR. The exact relation between the two phenomena awaits further investigation.

Hypertension SHR Brain Catecholamines 6-Hydroxydopamine Open field behaviour Rearing Locomotor activity

A growing amount of literature suggests that spontaneously hypertensive rats (SHR) exhibit behavioural and cardiovascular hyperreactivity [28]. Thus, when exposed to stress, SHR show larger changes in blood pressure and heart rate [11,18] and plasma catecholamines [15,20] when compared to normotensive Wistar-Kyoto rats (WKY). Furthermore, differences in pituitary-adrenal activity have been described [8,26] and in open-field experiments SHR display higher scores for locomotor and exploratory activity [14,19]. The hypertension which develops with age in SHR may result from the altered cardiovascular responses to environmental stimuli. The hyperreactivity may lead to increased pressor loads on the peripheral cardiovascular system and so induce structural changes contributing to a state of permanently elevated blood pressure [6].

The neurochemical substrate for either the behavioural changes or the high blood pressure in SHR is not clear. However, an important role for brain catecholamine systems in the development of hypertension in SHR is suggested by a number of reports concerning age-dependent changes in the levels or turnover of noradrenaline [5, 13, 37], dopamine [22,33] and adrenaline [24,36] in different brain areas of SHR when compared to WKY. Moreover, destruction of central catecholamine systems by intracerebroventricular (ICV) administration of 6-hydroxydopamine (6-OHDA) at the prehypertensive stage has been shown to inhibit the rise in blood pressure in SHR [9,29].

In view of the possible relation between behavioural changes and the development of spontaneous hypertension, the present experiments were carried out to investigate the effects of ICV administered 6-OHDA on behaviour of SHR. This could shed more light on the mechanism by which central catecholamine depletion by 6-OHDA treatment affects the rise in blood pressure. The results indicate that this treatment, in addition to its effects on blood pressure in the animals, induces selective alterations in spontaneous behaviour of SHR in response to the mild stress of environmental stimuli.

METHOD

Animals, Treatments

Male SHR-cpb, WKY-cpb [2] from TNO, Zeist, The Netherlands, were used. For comparison, in some experiments Wistar-cpb were used also. The animals were weaned at the age of four weeks and were kept in our laboratory under a constant light-dark rhythm (lights on 5.30 a.m., lights off 7.30 p.m.) with free access to standard pellet food and tap water. The rats were housed five in a cage except during the treatment period between the operations and training for blood pressure measurements.

At the age of four weeks, SHR and WKY were anesthestized with Hypnorm® and provided with bilaterally placed polyethylene cannulas in the lateral cerebral ventricles [1,29]. After a recovery period of 5–7 days, conscious animals were injected either with 6-OHDA, dissolved as 200

TABLE 1

SYSTOLIC BLOOD PRESSURE OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND WISTAR-KYOTO RATS (WKY) AFTER TREATMENT WITH 6-OHDA (FIRST INJECTION ON DAY 0, AGE 35 DAYS) OR VEHICLE, AND WISTAR RATS

	Blood Pressure	
	day 18	day 39†
SHR veh	188 ± 3‡	204 ± 3‡
SHR 6-OHDA	158 ± 3*‡	171 ± 4*‡
WKY veh	137 ± 2	139 ± 3
WKY6-OHDA	127 ± 3*	124 ± 2*
Wistars	132 ± 2	143 ± 2

For SHR and WKY n=14, for Wistars n=6.
*$p<0.05$ for difference between rats treated with 6-OHDA and vehicle.
†Age of the animals 53 days and 74 days respectively.
‡$p<0.05$ for difference between SHR and WKY.

μg base per 10 μl 0.9% saline with ascorbic acid (0.1 mg/ml), or the vehicle only. The first 10 μl was given through the right ventricular cannula and 48 hours later the second injection was administered through the left ventricular cannula. Again 48 hours later the third dose was given as two bilateral 5 μl injections. Thus ICV 6-OHDA treated rats received a total dose of 600 μg base [29]. This protocol is an adaption of the treatment introduced by Hauesler and co-workers [9]. After the third injection the rats were allowed a recovery period of three days after which training procedures were started for indirect measurements of blood pressure.

Wistar rats were not operated or injected but otherwise were used and maintained under identical conditions as the SHR and WKY.

Blood Pressure and Heart Rate

Systolic blood pressure was measured on conscious animals with a tail-cuff method as previously described [17]. SHR, WKY as well as Wistar rats were handled and trained daily for blood pressure determination before actual values were obtained. The measurements were performed at least three weeks thereafter during which the rats' blood pressure was measured at least three times a week. Care was taken not to heat-stress the animals.

Open-Field Testing and Animex

From previous experiments [29] it was clear that three to four weeks after the first 6-OHDA injection differences in the blood pressure and heart rate were established and non-specific side-effects of the treatment had disappeared. Behavioural tests were performed from this time-point on using a 'large' open field (especially for the measurement of ambulation and rearing), a 'small' open field (especially for ambulation and stereotyped behaviour), and an Animex activity meter.

The large open field consisted of a walled circular arena (80 cm diameter, 31 cm height) of which the floor was divided into oblong blocks with a 8 cm radius circle in the center. During the tests the room was illuminated only by a 60 W bulb approximately 50 cm from the floor of the open field. Ambulation score was determined as the number of

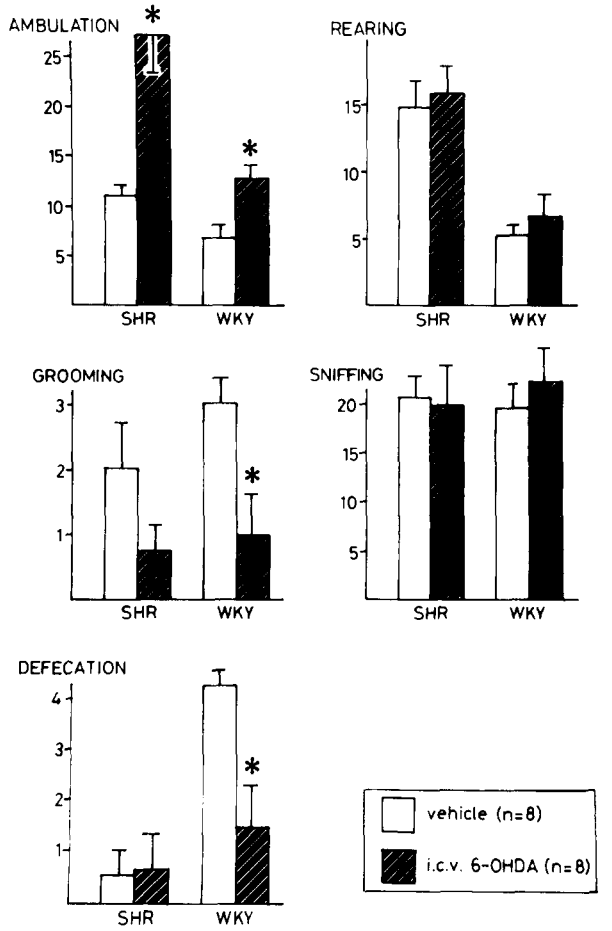

FIG. 1. The effect of ICV treatment with 6-OHDA on behaviour of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) in the small open field. For all groups n=9. *Refers to a statistically significant difference ($p<0.05$) between 6-OHDA and vehicle-treated rats. For other comparisons see text.

floor units crossed in the outer ring (ambulation outer) or in the inner ring including the center circle (ambulation inner). Two rearing scores were obtained. The rearing was considered to be rearing wall when the rat reared in the outer circle with the wall as support or the rearing was clearly directed towards the wall. Otherwise it was marked as rearing free. Defecation and the frequency and duration of grooming episodes were also scored [34]. The animals were tested during five minutes after placement in the center of the open field floor on three consecutive days.

The small open field consisted of a perspex circular test cage (diameter 19.5 cm, height 27.5 cm), the bottom of which was divided into four equal sections. Behavioural parameters scored for three minutes were ambulation (number of floor sections entered with all four paws), rearing, frequency of grooming, duration of stereotyped sniffing and defecation [32].

General motor activity was tested with an Animex activity meter [27] type DO (Farad Electronics, Stockholm, Sweden). The sensitivity was set at 10 μA for channel I and 40 μA for channel II for recording mainly large gross movements (locomotion) or all movements respectively. Activity was recorded for 20 minutes.

TABLE 2A

THE EFFECT OF CENTRAL ADMINISTRATION of 6-OHDA ON AMBULATORY ACTIVITY OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE WISTAR-KYOTO (WKY) AND WISTAR RATS IN THE LARGE OPEN FIELD

	Strain/Treatment				
Day	SHR veh	SHR 6-OHDA	WKY veh	WKY 6-OHDA	Wistars
Ambulation Total					
1	71.9 ± 5.1	75.4 ± 7.7	49.8 ± 4.4	49.0 ± 10.4	100.6 ± 12.2
2	68.4 ± 4.7	90.1 ± 12.1	49.7 ± 3.3	46.4 ± 9.5	71.5 ± 13.9
3	74.0 ± 7.2	95.4 ± 12.7	36.4 ± 4.4	39.3 ± 7.5	61.3 ± 10.9
Ambulation Outer					
1	51.4 ± 5.1	66.1 ± 7.6	39.4 ± 9.7	43.3 ± 9.7	87.2 ± 9.2
2	53.8 ± 3.4	80.9 ± 11.4	42.1 ± 3.7	41.4 ± 8.1	55.7 ± 10.2
3	58.4 ± 4.6	80.7 ± 11.5	31.5 ± 4.4	35.4 ± 6.7	50.8 ± 7.5
Amublation Inner					
1	20.5 ± 2.6	9.3 ± 1.2	10.5 ± 2.0	5.7 ± 1.4	13.5 ± 3.8
2	14.6 ± 2.6	9.1 ± 2.2	7.6 ± 1.2	5.0 ± 1.9	15.8 ± 4.2
3	16.4 ± 3.8	14.7 ± 3.6	4.8 ± 1.3	3.9 ± 1.4	10.5 ± 4.9

Animals were tested for 5 minutes on three consecutive days. For SHR and WKY groups n=14, for Wistar rats n=6. For statistical comparisons see text.

TABLE 2B

THE EFFECT OF CENTRAL ADMINISTRATION OF 6-OHDA ON REARING ACTIVITY OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE WISTAR-KYOTO (WKY) AND WISTAR RATS IN THE LARGE OPEN FIELD

	Strain/Treatment				
Day	SHR veh	SHR 6-OHDA	WKY veh	WKY 6-OHDA	Wistars
Rearing Total					
1	35.5 ± 3.7	7.3 ± 1.4	6.4 ± 1.2	5.6 ± 2.2	28.7 ± 7.3
2	29.4 ± 4.0	12.6 ± 4.7	8.4 ± 1.5	3.6 ± 1.3	10.7 ± 3.7
3	27.2 ± 4.3	8.6 ± 1.6	5.0 ± 0.8	2.1 ± 0.8	7.7 ± 2.4
Rearing Wall					
1	20.4 ± 2.3	7.2 ± 1.4	6.1 ± 1.2	4.8 ± 1.8	21.5 ± 4.9
2	20.0 ± 2.3	10.6 ± 3.6	7.5 ± 1.4	3.4 ± 1.3	8.3 ± 3.4
3	19.3 ± 2.6	7.6 ± 1.3	5.0 ± 0.8	2.0 ± 0.8	6.7 ± 1.8
Rearing Free					
1	15.1 ± 2.2	0.1 ± 0.1	0.2 ± 0.1	0.7 ± 0.4	7.2 ± 2.6
2	9.3 ± 2.2	2.0 ± 1.2	0.7 ± 0.1	0.1 ± 0.1	2.3 ± 1.1
3	7.8 ± 2.1	1.0 ± 0.5	0.0 ± 0.0	0.1 ± 0.1	1.0 ± 0.7

Animals were tested for 5 minutes on three consecutive days. For SHR and WKY groups n=14, for Wistar rats n=6. For statistical comparisons see text.

Catecholamines

For determining the effect of ICV 6-OHDA on brain catecholamine levels, the same animals were used as for the behavioural measurements.

Brain tissue was obtained after decapitation at 41 days after the first 6-OHDA injection (i.e., 1–2 weeks after behavioural testing). Brains were excised rapidly and dissected according to the method of Gispen *et al.* [7]. Tissue parts were weighed, frozen on dry ice and kept at −80°C for catecholamine assay.

Brain noradrenaline and dopamine concentrations were measured with a radio-enzymatic assay in homogenates of frontal cortex, hypothalamus and medulla-pons according to the method of Van der Gugten *et al.* [31].

Statistical Analysis

The effect of 6-OHDA treatment on behaviour of SHR and WKY in the large open field was evaluated with a repeated measures ANOVA [4] with two between groups factors (strain and treatment) and one repeated measure factor (day). In a separate analysis the SHR and WKY vehicle groups and the Wistar group were compared with a repeated

measures ANOVA with one between-group factor (strain) and one repeated groups factor (day). The other data were compared with one-way ANOVA and Duncan's multiple range test. A probability level of $p<0.05$ was considered statistically significant. Data are expressed as mean±standard error of the mean (S.E.M.).

RESULTS

Animals, Blood Pressure

During and directly after 6-OHDA treatment the rats showed increased irritability, an effect which wore off during the training period for blood pressure measurement. During the rest of the experimental period 6-OHDA-treated SHR and WKY were easier to handle than their respective vehicle-treated controls.

A slight and shortlasting decrease in body weight of 6-OHDA-treated rats was found after the first injection. Thereafter, all treatment groups showed parallel weight gain during the remainder of the experiment. Final body weight values, $F(3,52)=20.10$, $p<0.05$, were 265±5 and 225±8 g for vehicle-treated and 6-OHDA-treated SHR respectively ($p<0.05$) and 221±7 and 193±6 for vehicle-treated and 6-OHDA-treated WKY ($p<0.05$). A similar difference was observed in earlier studies [29,30].

Blood pressure data at 18 and 39 days after the first 6-OHDA injection are shown in Table 1. During the period of blood pressure measurement vehicle-treated SHR exhibited an increase in systolic blood pressure from around 170 mm Hg to a plateau exceeding 200 mm Hg. Vehicle-treated WKY had relatively stable blood pressure values around 140 mm Hg, while Wistar rats displayed values of approximately 135 mm Hg. Injection of 6-OHDA on days 0, 2 and 4 resulted in an inhibition of the development of hypertension in SHR as shown by markedly lower values of systolic blood pressure in this treatment group during the whole period of measurement. In WKY also a lower blood pressure was observed in the 6-OHDA treated rats, but this difference was smaller than that found between the SHR groups. Thus, at day 18, $F(3,52)=86.72$, $p<0.001$, and day 39 after treatment, $F(3,52)=145.43$, $p<0.001$, the differences between vehicle-treated SHR and WKY and between 6-OHDA-treated and vehicle-treated rats of either strain was significant.

Heart rate was lower after 6-OHDA treatment in both SHR and WKY. Heart rate values on day 39 after treatment, $F(3,52)=19.66$, $p<0.001$, were 432±8 and 361±10 in and 6-OHDA-treated SHR respectively ($p<0.05$), 459±10 and 394±7 B/min in vehicle- and 6-OHDA-treated WKY respectively ($p<0.05$). Further details concerning heart rate effects of 6-OHDA treatment have been reported previously [29,30].

Open-Field and Animex Tests

Results from the small open field are shown in Fig. 1. Ambulation scores, $F(3,28)=15.60$, $p<0.001$, showed a significant increase in ambulatory activity after 6-OHDA-treatment in both SHR and WKY. Rearing, $F(3,28)=11.45$, $p<0.001$, was higher in SHR but treatment with 6-OHDA had no significant effect here. Grooming score, $F(3,28)=3.73$, $p=0.023$, was significantly decreased in WKY after 6-OHDA-treatment. The decrease in SHR here was not significant. Sniffing scores, $F(3,28)=0.124$, showed no significant differences between the groups. Defecation, $F(3,28)=11.42$, $p<0.001$, was significantly higher in vehicle-treated WKY when compared to vehicle-treated SHR. After 6-OHDA-treatment defecation was decreased in WKY.

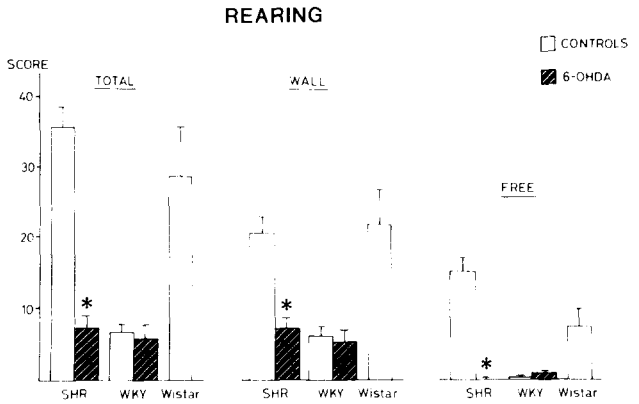

FIG. 2. The effect of ICV treatment with 6-OHDA on rearing behaviour of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) and Wistar rats in the large open field. *Refers to a statistically significant difference ($p<0.05$) between 6-OHDA and vehicle-treated rats. See also Table 2.

Scores of the large open field are summarized in Table 2 and Fig. 2. Statistical comparison concerning the effect of 6-OHDA does not include the Wistar group. Ambulatory activity of SHR and WKY was little influenced by 6-OHDA treatment. Although a significant effect of Strain, $F(1,52)=25.53$, $p<0.001$, was found for total ambulation as well as for ambulation outer, $F(1,52)=17.47$, $p<0.001$, and ambulation inner $F(1,52)=18.50$, $p<0.001$, separately, only with ambulation inner an effect of Treatment was found, $F(1,52)=6.15$, $p=0.016$, and no significant Strain-Treatment interactions occurred. Thus, SHR and WKY differ in their ambulatory activity in the large open field, but the eventual effects of 6-OHDA treatment occur in a comparable way in the two strains. Habituation was not found as indicated by the absence of an effect of Day. A Day-Strain interaction ($F(2,104)=5.00$, $p=0.008$ for ambulation total, $F(2,104)=4.31$, $p=0.016$ for ambulation outer) was found, however, which may reflect a tendency for ambulation to increase in SHR groups over repeated testing as compared to a decrease in WKY groups.

More clear effects were observed on rearing activity in the large open field. Not only was an effect of strain observed on all parameters measured ($F(1,52)=54.23$, $p<0.001$ for rearing total, $F(1,52)=46.98$, $p<0.001$ for rearing free and $F(1,52)=33.46$, $p<0.001$ for rearing wall), but also an effect of Treatment ($F(1,52)=34.68$, $p<0.001$, $F(1,52)=26.06$, $p<0.001$ and $F(1,52)=24.21$, $p<0.001$ respectively). Moreover, a significant Strain-Treatment interaction indicated differential effects of 6-OHDA treatment on rearing behaviour ($F(1,52)=20.42$, $p<0.001$, $F(1,52)=10.03$, $p=0.003$ and $F(1,52)=25.21$, $p<0.001$ respectively), reflecting a decrease in rearing activity (all three parameters) after 6-OHDA in SHR only. In addition, an effect of Day was only found in values for rearing free, $F(2,104)=5.15$, $p=0.015$, which, combined with a Day-Treatment interaction, $F(2,104)=6.70$, $p=0.005$, and a Day-Strain-Treatment interaction, $F(2,104)=9.66$, $p=0.001$, may be explained by a decrease of rearing scores of vehicle-treated SHR only. A Day-Strain-Treatment interaction was also found for rearing total, $F(2,104)=3.41$, $p=0.043$ (Table 2).

Grooming activity was lower in 6-OHDA treated rats

TABLE 3

ANIMEX SCORE OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND NORMOTENSIVE WISTAR-KYOTO RATS (WKY) AFTER ICV TREATMENT WITH VEHICLE OR 6-OHDA

	Gross	Total	n
SHR veh	710 ± 47	1662 ± 100	4
SHR 6-OHDA	859 ± 41	2015 ± 94	4
WKY veh	305 ± 35†	1110 ± 79†	3
WKY 6-OHDA	681 ± 49*	1813 ± 124*	3

Large gross movements (locomotion) were measured by the low-sensitivity channel; total movements were measured by the high-sensitivity channel. Activity was recorded for 20 minutes.

*$p<0.05$ for difference between 6-OHDA and vehicle-treated rats.

†$p<0.05$ for difference between SHR and WKY.

TABLE 4

BRAIN CATECHOLAMINE CONCENTRATION OF SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE WISTAR-KYOTO RATS (WKY) AFTER ICV TREATMENT WITH 6-OHDA

	SHR veh	SHR 6-OHDA	WKY veh	WKY 6-OHDA
Noradrenaline				
Frontal cortex	256 ± 27	5 ± 2*	179 ± 19	18 ± 6*
Hypothalamus	807 ± 144	351 ± 35*	1006 ± 175	358 ± 34*
Medulla/pons	420 ± 67	144 ± 25*	274 ± 16	125 ± 16*
Dopamine				
Frontal cortex	368 ± 95	128 ± 31*	192 ± 47†	71 ± 6*
Hypothalamus	349 ± 75	330 ± 49	321 ± 57	348 ± 44
Medulla/pons	70 ± 19	42 ± 7	49 ± 7	67 ± 6

*$p<0.05$ for difference between 6-OHDA and vehicle-treated rats.

†$p<0.05$ for difference between SHR and WKY.

Brain tissue was obtained after decapitation at 41 days after treatment (age 76 days). Concentration of noradrenaline and dopamine expressed in pg/mg tissue wet weight.

(Treatment-effect, $F(1,52)=6.68$, $p=0.013$), but the absence of an effect of Day or of an interaction between Strain and Treatment, indicates that this effect is comparable for both SHR and WKY. Defecation scores revealed a decrease after 6-OHDA in WKY with no effect in SHR (Strain-effect, $F(1,52)=29.79$, $p<0.001$, Treatment-effect, $F(1,52)=4.09$, $p=0.048$ and Strain-Treatment interaction, $F(1,52)=10.52$, $p=0.002$).

Comparison of data from vehicle-treated SHR and WKY and Wistar rats in a separate ANOVA revealed a general absence of habituation in SHR as well as WKY but not in Wistars. Thus a significant Day-Strain interaction was found for ambulation-outer, $F(4,62)=11.42$, $p<0.001$, ambulation-total, $F(4,62)=6.18$, $p<0.001$, rearing wall, $F(4,62)=4.05$, $p=0.006$, rearing free, $F(4,62)=4.23$, $p=0.010$, and rearing total, $F(4,62)=3.80$, $p=0.008$, reflecting the decrease in scores for these parameters found in Wistar rats over the three days of measurement, with no changes in SHR and WKY (Table 2).

Total 20 minute Animex scores are shown in Table 3. For both gross movements, $F(3,10)=13.00$, $p<0.001$, and total activity, $F(3,10)=9.86$, $p<0.003$, the difference between vehicle-treated SHR and WKY and between 6-OHDA-treated and vehicle-treated WKY was significant. A tendency for increased activity after 6-OHDA-treatment was found in SHR also, however.

Brain Catecholamines

The effect of ICV administered 6-OHDA on brain catecholamine concentrations is shown in Table 4. In general depletion patterns were the same in SHR and WKY. Significantly lower noradrenaline levels were found in frontal cortex (>90% depletion; $F(3,28)=60.79$) and in hypothalamus and medulla-pons (>50%; $F(3,28)=8.99$ and 14.98 respectively). The concentration of dopamine in frontal cortex, $F(3,28)=5.95$, $p<0.05$, was significantly higher in control SHR when compared to control WKY (see also [30]). The depletion of dopamine caused by 6-OHDA was comparable in both strains (approximately 60%). No differences were observed between the groups in dopamine concentration in the hypothalamus, $F(3,28)=0.04$, and medulla-pons, $F(3,28)=1.76$.

DISCUSSION

The present experiments were performed to investigate whether ICV administration of 6-OHDA at the prehypertensive stage, in addition to its inhibiting effect on the development of hypertension, induces specific changes in spontaneous behaviour of SHR when compared to similarly treated WKY. The results indicate that especially rearing activity in the large open field is affected. In general, other parameters were either not affected (e.g., stereotyped sniffing) or changed in a comparable way in SHR and WKY (ambulation, activity in Animex, grooming). Thus, the disruption of central catecholamine systems does not alter the tendency for SHR to show higher ambulation in the open field as compared with similarly treated WKY.

Behaviour of SHR vs. WKY: Controls

Behaviour of SHR is characterized by hyperreactivity to environmental stimuli. Although employing different protocols, open field experiments by McCarty and co-workers as well as by Knardahl and Sagvolden showed higher locomotor activity as well as higher exploratory activity of SHR when compared to normotensive WKY [14,19]. When compared to other normotensive strains, however, SHR were found to show no differences in these parameters [21]. Little habituation to the test environment was found in SHR [23]. The results of our present experiments support these findings. When compared to WKY, SHR consistently displayed higher scores for ambulation and rearing in the open field, which could suggest higher locomotor activity and exploration behaviour respectively. Grooming scores were not different but SHR showed less defecation. The latter finding may support the hypoemotionality ascribed to SHR by others using classical conditioning experiments [16,25].

In our experiments habituation was found neither in SHR nor in WKY. An additional control group of Wistar rats did show clear habituation, however. Together with the fact that

ambulation and rearing levels in SHR are close to the values found in Wistars, this indicates that care has to be taken which strain is chosen as controls for the SHR. Because of their close genetical relationship to SHR, WKY are generally considered the best control-strain for these animals [2,28].

Behaviour of SHR vs. WKY: The Effect of 6-OHDA

The most pronounced effecct of ICV 6-OHDA treatment, in addition to the attenuation of the development of hypertension, was on rearing in the large open field. Surprisingly, rearing in the small open field was not affected by ICV 6-OHDA. This might be explained by the size of the open field and the differences in behavioural repertoire of the rats in these experiments. Rearing in the small open field may be more strongly influenced by ambulatory activity, thus obscuring treatment effects on 'exploratory' rearing. Hardly any rearing-free is observed in the small open field. McCarty *et al.* have also reported the behavioral differences between SHR and WKY to vary in relation to the kind of stimulus used [18].

Defecation was decreased in WKY but not in SHR. The level of defecation in SHR is already very low. Nevertheless, if this parameter is considered indicative of the level of fear or emotionality [3] this could mean that these behavioral characteristics are unrelated to hypertension. Thus, the largest change in defecation was found in the group with the smallest blood pressure effect (WKY). However, higher activity scores have also been suggested to relate to high emotionality [3]. This parameter is not changed similarly to defecation. For determining the level of fear and emotionality, other experimental paradigms may be more valuable (see [16,25]).

Behaviour and Hypertension

The changes in behaviour in SHR when compared to WKY have been suggested to be related to the development of hypertension in this strain [10,28]. One approach to study this relation is the use of treatments which affect the development of hypertension. When such a treatment causes changes in behaviour of SHR towards levels found in WKY, behaviour and blood pressure may be related. Behavioural changes which occur in a comparable way in SHR and WKY after an antihypertensive treatment do not support the suggestion of a relation between blood pressure and hypertension. Thus, in the present study ambulation after 6-OHDA-treatment was either not changed (large open field) or increased (small open field, Animex) in both SHR and WKY. In contrast, rearing was decreased only in SHR. Acute and chronic treatment with hydralazine to lower blood pressure in SHR has been shown not to affect locomotor behaviour either [35]. Also, in WKY and Sprague-Dawley rats made hypertensive by renal clips, no effects on locomotor activity were found [35]. Recently, also through backcrossing a separation of the increased locomotor activity of SHR and the development of hypertension was found [12]. These results suggests that locomotor activity and hypertension are not linked and that certain treatments may affect either one of them in a different way as also appears from our results. Because the above mentioned authors used an automated activity cage in their experiments, the relation between explorative behaviour and the development of hypertension could not be measured in their experiments. Rearing activity was changed only in SHR in the large open field in our experiments. In addition, SHR showed a clear attenuation of the development of hypertension by 6-OHDA as opposed to only a small decrease in systolic blood pressure in WKY. However, as outlined by Tucker and Johnson, even in this case different correlations between hypertension and behaviour are possible [28]. Thus, the relation can be either pleiotropic (i.e., both hypertension and changes in behaviour may be caused by a common genetical defect), causal (hypertension is caused by behavioural changes or causes them) or non-causal (hypertension and behavioural changes are caused by separate defects or separate genes due to random fixation during breeding). It is obvious that care has to be taken before effects on both blood pressure and behaviour can be attributed to either one of the relations. Further experiments will be needed to clarify this. For instance, the observation that antihypertensive treatment of SHR with hydralazine did not change either open field ambulation or rearing ([35], Van den Buuse *et al.*, submitted) may indicate that changes in blood pressure per se are not responsible for effects on behaviour.

The specificity of the changes in rearing activity together with the large effects on blood pressure thus warrant further investigation to correlate the effects of other treatments in the catecholamine systems of SHR on blood pressure and behaviour. Especially more specific lesions may be important here, since both noradrenaline and dopamine (and also adrenaline, see [29]) were depleted in different structures of the brain (and the spinal cord, see [30]) after 6-OHDA treatment. Recently, we showed that brain dopamine rather than noradrenaline may be of major importance for the rise in blood pressure in SHR [30]. Further comparison of the effects of lesions may give more insight regarding the brain neurotransmitter systems involved in the development of hypertension and about the behavioural aspects in this process.

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