Neuroscience Letters, 77 (1987) 71–75
Elsevier Scientific Publishers Ireland Ltd.

NSL 04595

Grooming behavior of spontaneously hypertensive rats

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(Received 30 December 1986; Revised version received and accepted 2 February 1987)

Key words: Grooming; Hypertension; ACTH; Open field; Rat

In an open field spontaneously hypertensive rats (SHR) exhibited lower scores for grooming when compared to their normotensive controls, the Wistar–Kyoto rats (WKY). After i.c.v. injection of 1 μg $ACTH_{1-24}$ cumulative 50-min grooming scores were lower in SHR. Analysis of subscores indicated that the lesser effect of ACTH in SHR was especially prominent for headwashing and anogenital grooming. Moreover, a time course study revealed that the difference between SHR and WKY occurred in the first 6 observation periods of 5 min and thereafter disappeared. The results are discussed in the light of behavioral and central neurochemical differences between SHR and WKY.

Spontaneously hypertensive rats (SHR) have been extensively used to study possible mechanisms in the development of genetic hypertension [3, 11]. A number of investigators have reported behavioral changes in this strain [7, 8, 10]. For instance, when opposed with a more or less stressful situation, SHR show exaggerated hemodynamic and humoral responses. The behavioral changes in SHR have often been summarized as hyperreactivity to stress [8, 10]. A relation between this behavioral characteristic and the development of hypertension has been suggested [3, 8, 10, 13]. A number of central treatments affect both behavior and the development of hypertension in SHR [3, 4, 13, 15]. For instance, central treatment with the catecholamine neurotoxin 6-hydroxydopamine or lesions in the brain nigrostriatal system induce an attenuation of the rise in blood pressure with age [12, 14]. Such treatments also normalize the exaggerated responses of SHR in an open-field test [13, 15].

Little is known about grooming behavior in SHR when compared to their normotensive controls, the Wistar–Kyoto rats (WKY). In the rat, grooming activity can be increased by various stressors, including a novel environment. Also a number of drugs can elicit excessive grooming. Especially the central injection of ACTH is well

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known in this respect [5, 6]. Central dopamine systems play an important role in the regulation of novelty- or ACTH-induced grooming [2, 5, 9, 17, 18]. In view of the above-mentioned postulated role of central dopamine in hypertension in the SHR, the present study is focussed on grooming behavior in SHR and WKY.

Male SHR and WKY were used. The animals were 7–8 weeks of age at the time of the experiments and were used only once. The rats were housed under a standard light–dark rhythm with food and water available ad libitum.

Open-field activity and grooming was monitored in an unoperated group of naive SHR and WKY. The animals were tested in an 80-cm diameter open field during 5 min [13]. Lighting was provided by a 60 W bulb approximately 50 cm above the floor of the open field. Grooming was scored in 5-s bouts. Thus, when the animal would be grooming continuously for 15 s this would result in 3 grooming scores. If the animal would groom for 3 s this would result in 1 grooming score. Ambulation was scored as the number of floor units crossed. Rearing was counted when the animal stood stretched on its hindpaws. For a defecation score the number of fecal boli remaining in the open field after the 5-min test was counted.

For the measurement of ACTH-induced grooming, the rats were anesthetized with Hypnorm (10 mg fluanison, 0.2 mg fentanyl/ml, 1 ml/kg), one week before the experiments and provided with a polyethylene cannula in the lateral cerebral ventricle [1]. ACTH-induced grooming was assessed according to Gispen et al. [5, 6]. Briefly, the animals were injected with 1 μg $ACTH_{1-24}$ in 2 μl of artificial cerebrospinal fluid. Control groups from either strain were injected with the vehicle only. Immediately after the injection, the rats were placed in clear perspex observation boxes ($26 \times 20 \times 13$ cm). Using a 15-s sampling time different grooming elements were scored for a total period of 50 min starting 10 min after the central treatment. Total grooming was calculated as the sum of the following elements: head washing, paw licking, body grooming, anogenital grooming and scratching [6, 17]. A time curve of the effect of ACTH was derived by using total ACTH-induced grooming scores from subsequent individual 5-min periods. Scores of the various elements or basal grooming scores in vehicle-treated rats were too low for reliable statistical analysis of time effects. For the present data, the results of 3 identical experiments were pooled.

Data are expressed as mean $\pm$ S.E.M. The data were analyzed by two-way analysis of variance (ANOVA) with strain and treatment as factors. A between-group comparison was performed by Duncan's multiple range test. The time curve of the effect of ACTH was analyzed by analysis of variance for repeated measures (MANOVA) with time as within-subjects factor and strain as between-subjects factor.

In the open field SHR displayed significantly lower scores for grooming when compared to WKY. Ambulation and rearing scores were increased, while defecation was lower (Table I).

Table II summarizes grooming activity of SHR and WKY after central injection of 1 μg $ACTH_{1-24}$ or vehicle. ANOVA indicated an overall strain difference for total grooming score, head-washing and anogenital grooming. An overall effect of the ACTH treatment was found for total score, head washing, body grooming and paw

TABLE I

OPEN-FIELD BEHAVIOR OF SHR AND WKY

*$P<0.05$ for difference between SHR and WKY. Data are mean ± S.E.M. For both groups: $n = 9$.

	Ambulation	Rearing	Grooming	Defecation
WKY	65.9 ± 6.6	6.9 ± 1.8	6.9 ± 1.4	3.3 ± 0.6
SHR	86.6 ± 7.6*	18.7 ± 3.0*	2.9 ± 1.0*	0.4 ± 0.2*

licking. No statistical significant interaction was observed between strain and treatment, indicating that ACTH had a similar effect in both SHR and WKY. Between-group analysis indicated a significant increase in grooming with respect to total score, paw licking, head washing and body grooming in SHR as well as in WKY after ACTH treatment. Scores of the different grooming elements were always lower in SHR after ACTH treatment than in WKY. A significant strain difference in ACTH-treated animals was found for total grooming, head washing and anogenital grooming. Saline-treated SHR and WKY did not differ in grooming scores, although total score, head washing and anogenital grooming tended to be lower in SHR. In pilot experiments, the above-mentioned differences between SHR and WKY were also found when 2 μg of ACTH were injected instead of 1 μg (data not shown).

When the total grooming score after ACTH treatment was plotted as a function of time after the injection (Fig. 1), WKY showed significantly higher values during the first 6 observation periods (5 min each). MANOVA indicated that the overall strain difference was significant. Neither a significant time effect, nor a significant interaction of strain and time was found. This indicates that the time course of the effect of ACTH is essentially similar in SHR and WKY. However, WKY showed a tendency towards a decrease in scores from period 5 to period 12. This could explain the lack of a strain difference in the last 4 observation periods.

TABLE II

THE EFFECT OF I.C.V. INJECTION OF 1 μg $ACTH_{1-24}$ ON GROOMING ACTIVITY OF SHR AND WKY

*$P<0.05$ for difference between SHR and WKY; **$P<0.05$ for difference between ACTH-treated and saline-treated rats from the same strain. Data are mean ± S.E.M.; n, the number of animals in each group.

	WKY–vehicle	SHR–vehicle	WKY–ACTH	SHR–ACTH
Total score	45.0 ± 6.3	39.4 ± 7.6	123.1 ± 7.1**	94.8 ± 8.1*,**
Head washing	14.1 ± 2.8	7.9 ± 1.2	34.5 ± 2.9**	24.9 ± 3.6*,**
Body grooming	13.2 ± 2.2	15.9 ± 3.8	54.1 ± 5.6**	47.3 ± 8.7**
Anogenital gr.	6.1 ± 1.3	3.7 ± 0.7	7.9 ± 1.1	3.5 ± 0.8*
Paw licking	4.0 ± 0.9	4.6 ± 1.0	14.3 ± 1.7**	10.4 ± 1.9**
Scratching	7.6 ± 2.1	7.3 ± 2.5	12.3 ± 2.3	8.7 ± 2.6
n	16	15	24	15

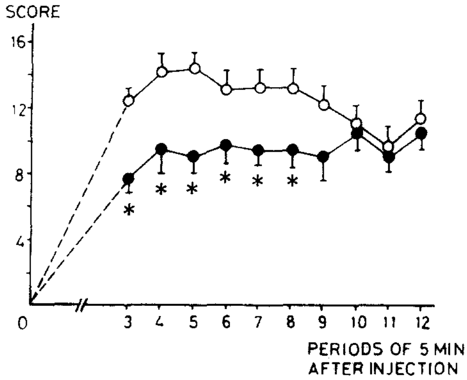

Fig. 1. Time-course of ACTH-induced excessive grooming in SHR (closed circles) and WKY (open circles). The number of animals is 15 and 24 respectively. MANOVA indicated a significant overall strain difference (see text). $*P<0.05$ for strain difference at individual time points (Duncan's multiple range test).

Differences in behavior between SHR and WKY have been reported previously (for a review see refs. 3, 8, 10). After a variety of more or less stressful situations, SHR show exaggerated behavioral responses. Thus, SHR are hyperreactive to handling, light, heat and noise [8, 10]. In the open field, higher scores for ambulation and rearing were found [7, 13, 15], as also shown in the present study. In those studies, grooming activity and defecation were reported to be lower. Vehicle-treated SHR and WKY in the ACTH test did not show a statistically significant difference in grooming activity. This result seems at variance with the lower open-field grooming found in SHR, and suggests that basal grooming activity is different between the strains only under certain circumstances. The increased exploratory activity of SHR in the open field could influence their grooming activity. Alternatively, the open field could induce a level of arousal in the animals different from that of the observation cages of the ACTH test [5, 6, 9], and thus result in a strain difference in the former test, but not in the latter. Further experiments are needed to study these possibilities.

The behavioral differences between SHR and WKY may reflect changes in the central relay of environmental input via the limbic system to brainstem centers involved in the regulation of autonomic functions [3, 4, 10, 13]. Exaggerated changes in blood pressure and heart rate have been described in SHR [3, 8]. Such an 'overstimulation' of the peripheral cardiovascular system could ultimately lead to structural changes in the vascular wall and thus lead to a permanently elevated blood pressure level [3].

The present results show another example of differences in behavior between SHR and WKY. Grooming behavior may be stimulated by a stressful situation (e.g. novelty). The differences in behavioral reactivity between SHR and WKY could thus be reflected also in their grooming activity.

The neurochemical substrate of grooming behavior remains to be elucidated. It is now well established, however, that central dopamine systems play a modulatory role in ACTH-induced grooming [2, 5, 17, 18]. Thus, the effect of ACTH was also found when the peptide was injected into the area of the substantia nigra [9]. It is interesting to note that in SHR, brain dopamine systems have been implicated in the development of hypertension [12, 14, 16]. This influence appears to be concentrated in the nigrostriatal system [14]. The presently observed lower grooming scores in the open field and after treatment with ACTH could be a reflection of changes in central dopaminergic function in SHR.

M.v.d.B. was supported by The Dutch Heart Foundation. The authors are grateful to J.C.J. Tombrock and S.K. Mangal for excellent technical assistance and to Dr. Tj.B. van Wimersma Greidanus for critically reading the manuscript.

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