Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, No. 4, pp. 789–794, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00034-8

Catecholamine Stimulation and the Response to Behavioral Challenge in Wistar Rats

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Received 18 May 1994

HALLER, J. *Catecholamine stimulation and the response to behavioral challenge in Wistar rats.* PHARMACOL BIOCHEM BEHAV **51**(4) 789–794, 1995.—Male Wistar rats were injected with CH-38083, an α_2-adrenoceptor blocker, after which they were challenged by a size-matched Wistar or Long-Evans opponent. In residents facing low-aggression opponents, the α_2-adrenoceptor blockade significantly reduced aggressiveness, whereas in those facing highly aggressive opponents the treatment significantly increased aggression scores compared to saline-treated controls, irrespective of the strain of the intruder. When the animals were treated with CH-38083, the frequency of biting attacks correlated significantly with the aggressiveness of the opponent in residents fighting with Wistar and Long-Evans rats. Similar correlations were not found in control (saline-injected) rats. The results suggest that the catecholaminergic activation caused by the α_2 receptor antagonist elicits a more efficient adaptation to the behavioral actions of the opponent. Plasma corticosterone levels were not influenced by the treatment, but this variable seemed to be correlated with the defensive behavior performed by the intruder.

Aggression Catecholamines α_2 Adrenoceptors Corticosterone Challenge Rat

ACTIVATION of the catecholaminergic system is one of the processes that parallels aggressive behavior. An aggressive encounter between previously isolated animals elicits a very rapid increase in catecholamines in both the CNS (5,17) and the periphery (10,11,21,25). The question arises whether this process is involved in the further modulation of aggressive behavior or if it is a simple consequence of the encounter. If the former assumption is true, the modulation of the catecholaminergic system previous to the aggressive encounter may modify the aggressive behavior of animals. There are several papers that show this effect can be obtained; however, the results are contradictory: both stimulation and inhibition of the adrenergic system appear to be stimulatory with respect to the agonistic behavior of animals (1).

Previously we hypothesized that the discrepancy between the results may be caused by methodological problems, because the methods of modulating the adrenergic system described in the review by Bell and Hepper (1) are not sufficiently selective. Most of the methods used at that time directly interfered with more than one neurotransmitter system. The more recent development of highly selective α_2-adrenoceptor antagonists offers a new way of studying the involvement of catecholamines in aggression. These substances remove the presynaptic negative feedback control of norepinephrine release, thus emphasizing naturally occurring adrenergic activation rather than inducing a catecholamine release by themselves (14,22,23,27). Thus, their actions are more "physiological" then those of other interventions.

In a previous experiment we have shown that different α_2-adrenoceptor blockers (yohimbine, idazoxan, and CH-38083) induce a several-fold increase of aggression in isolated Long-Evans rats (13). We have obtained bell-shaped dose-response curves with yohimbine and idazoxan, and a dose-dependent increase in CH-38083 in the range 0.5–2 mg/kg. In that experiment the baseline levels of aggression were very low: only 30% of controls fought when facing an intruder, and even these fought only one to two times as much (mean $\pm$ SE was 0.62 ± 0.042; $n = 9$).

Previous experience shows that Wistar rats behave more aggressively than Long-Evans rats. The present experiment is aimed at studing the effects of CH-38083 [7,8-(methylene dioxy)-14-α-hydroxyalloberbane; an α_2-adrenoceptor blocker] on the behavior of resident Wistar rats.

It was shown that aggressive encounters induce a marked increase in plasma corticosterone levels (20). In the above-mentioned previous experiments, we have shown that the α_2-adrenoceptor antagonist CH-38083 caused an increase in the corticosterone response to aggression: the treated animals showed a larger increase in plasma corticosterone levels compared to untreated fighters (13). It would be interesting to see whether this effect also occurs in animals with a higher baseline level of aggression.

The questions asked in the present experiment were: (i) does catecholaminergic activation increase the aggressiveness of animals even when the baseline levels of aggression are very high?; (ii) does the challenge received from the intruder influence the behavioral effects of the treatment?; and (iii) are there some links between the treatment, the aggressiveness of the opponent, and the stress response to aggression?

METHOD

Animals and Experimental Design

Male Wistar rats weighing approximately 400 g were used as experimental animals. The animals were fed on pelleted rat food ad lib, water was freely available, temperature was held constant at 23 ± 1°C, a 14L : 10D cycle was maintained, and humidity was held at 60 ± 10%. Animals naive to experimentation were used; each animal (including intruders) was used only once.

The experimental (resident) animals were housed in male-female pairs for approximately 2 weeks prior to the experiment. The pairs were transferred to the experimental cages (60 × 50 × 50 cm) 3 days prior to the experiment. All walls except the front wall of the cages were made of an opaque plastic; the front wall was transparent.

Half an hour before the start of the experiment, the females were removed from the cages. The animals were injected either with 1 mg/kg CH-38083 (Chinoin, Budapest, dissolved in physiological saline) or with physiological saline alone. Twenty minutes later an opponent was placed in the home cage of the treated animals for 15 min. Size-matched, group-housed Wistar or Long-Evans rats were used as intruder opponents in aggressive encounters. Thus, four groups were used: saline-injected Wistar residents were paired either with Wistar or with Long-Evans intruders (groups W × W saline and W × LE saline), and the same pairing with CH-38083-treated male rats (groups W × W CH-38083 and W × LE CH-38083). The treatments were randomized.

The behavior of the animals was videorecorded through the transparent front wall of the cages. After 15 min, the animals were killed by decapitation, and blood was collected for corticosterone measurements.

Behavioral Variables

The classical ethological work of Grant and Macintosh (8) defined the behavioral nomenclature used in the experiment.

The behavior of animals was videorecorded for 15 min; the recordings were later analyzed by means of an event recorder.

The following behavioral variables were measured:

- The duration of exploration (sniffing movements directed towards the walls of the cage, towards the floor, and in the air);
- the duration of social investigation (sniffing movement directed towards the flanks, the nose, and the anogenital region of the opponent);
- the frequency of bites and clinch fighting (rapid biting of the opponent without wrestling and very rapid rolling, jumping, and biting of both animals that are in close contact, respectively); the sum of the two variables is referred to as "biting attacks";
- the duration of offensive aggression (sum of aggressive grooming, lateral threatening, chasing, mutual upright, mounting, and keeping down); the total duration of these behaviors is referred to as "agonistic interactions";
- the duration of defensive behavior (sum of immobility, defensive upright, fleeing, and submissive posture).

The behaviors of both the resident (treated) and the intruder were recorded.

Corticosterone Assays

Corticosterone was measured in plasma without extraction. The antiserum was raised in rabbit against corticosterone-carboxymethyloxime-BSA. ^{125}I-Labelled corticosterone-carboxymethyloxime-tyrosine-methyl ester was used as tracer. The interference of plasma transcortin was eliminated by inactivating transcortin at low pH. The sensitivity of the assay was 0.1 pmol (19).

Statistical Evaluation

The statistical significance of differences in behavior was analyzed using the Kruskal-Wallis nonparametric test followed by Mann-Whitney pairwise comparisons; the results of hormonal determinations were submitted to ANOVA followed by REGWF pairwise comparisons. The Spearman test was used for the evaluation of correlations between different variables.

RESULTS

Contrary to expectations, Wistar intruders were not significantly more aggressive than Long-Evans intruders. Although a relatively large difference occurred [the percent of time spent with agonistic interactions was 5.28 ± 1.73 (n = 19) and 2.95 ± 1.13 (n = 21) in Wistar and Long-Evans intruders, respectively], the large variability made the difference nonsignificant. However, the behavior of the two strains was different: the percent of time spent with defensive behaviors was 15.75 ± 3.93 and 62.6 ± 4.02 in Wistar and Long-Evans intruders, respectively, $H(1) = 23.94, p < 0.0001$.

When grouped according to the strain of the intruder, the behavior of the control (saline-injected) and treated rats showed only minor differences (Table 1). Social behavior as well as exploration significantly increased in CH-38083-treated rats facing Long-Evans intruders compared with their saline-treated counterparts. A similar difference did not occur in rats facing Wistar opponents. Attack latency was very low in saline-treated residents facing Long-Evans rats. The attack latencies in the other three groups were similar (Table 1).

However, in the CH-38083-treated animals significant correlations were found between the time spent in agonistic behavior by the intruder and the number of biting attacks performed by the resident (Figs. 1 and 2). Similar correlations in the control rats did not occur (Figs. 1 and 2). This correlation raised the possibility that the effect of CH-38083 might depend on the behavior of the opponent.

The histogram of the frequency distribution of intruders in relation to their aggressiveness shows that the animals can be divided in two distinct groups (Fig. 3): a low-aggression group that spent less then 2% of the time in agonistic behavior, and a high-aggression group that engaged in agonistic behavior for more then 5.1% of the total time. Low-aggression intruders mounted a weak challenge to the resident, whereas the high-aggression intruders challenged the resident more powerfully. When the response given by the control and CH-38083-treated groups was grouped according to the challenge of the intruder, an interesting picture emerged (Fig. 4). Saline-treated controls totally ignored the intensity of challenge received: they performed the same number of attacks to both low- and high-

TABLE 1

THE EFFECTS OF CATECHOLAMINERGIC ACTIVATION ON THE BEHAVIORAL PARAMETERS IN WISTAR RESIDENTS FACING WISTAR OR LONG-EVANS INTRUDERS

Variable	W × W Saline (n = 9)	W × W CH-38083 (n = 10)	W × LE Saline (n = 10)	W × LE CH-38083 (n = 12)
Attacks	7.66 ± 2.49	12.20 ± 3.24	12.90 ± 3.26	5.66 ± 1.31
Bites	4.77 ± 1.53	9.20 ± 2.45	7.60 ± 1.93	3.42 ± 0.94
Clinch	2.88 ± 1.09	3.00 ± 0.96	5.30 ± 1.75	2.25 ± 0.71
Attack latency	357.8* ± 100.5	306.9* ± 108.7	79.18† ± 21.0	236.6*† ± 85.6
Agonistic interactions	24.4 ± 6.94	28.2 ± 7.83	28.8 ± 5.1	16.4 ± 4.34
Social investigation	17.26*† ± 2.82	12.46* ± 2.23	12.04* ± 1.89	21.64† ± 2.56
Exploration	27.27*† ± 4.10	29.51*† ± 4.64	19.42* ± 2.47	34.76† ± 4.39

Values (mean ± SE) are expressed in counts/15 min for attacks, bites, and clinch; in percent of time for agonistic interactions, social investigation and exploration; and in seconds for attack latency. Values labelled with different symbols are statistically different at the $p < 0.05$ level.

aggression intruders. In contrast, residents submitted to the α_2-adrenoceptor blockade were significantly less aggressive when faced with low-aggression intruders than their saline-treated counterparts, while being significantly more aggressive when faced with high-aggression intruders compared to controls (Fig. 4). Other behavioral variables showed statistically nonsignificant variations (data not shown).

Plasma corticosterone was significantly lower in rats facing Long-Evans intruders compared with the residents facing Wistar opponents (Fig. 5). However, when the results were grouped according to the aggressiveness of the intruders (ignoring the strain to which they belong), there were no differences in the corticosterone response of the residents: the intensity of challenge did not influence the corticosterone response to aggression. No differences between saline-treated and CH-38083-treated animals were found.

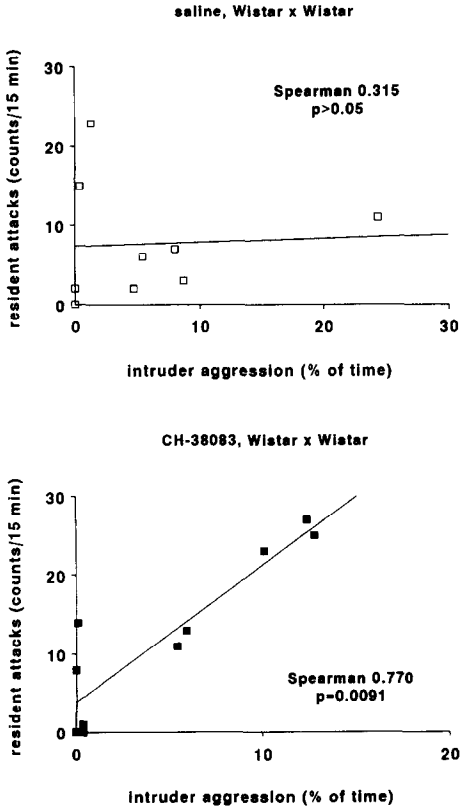

FIG. 1. The correlations existing between the aggressiveness of the intruders and the frequency of the resident's biting attacks in W × W saline and W × W CH-38083 groups.

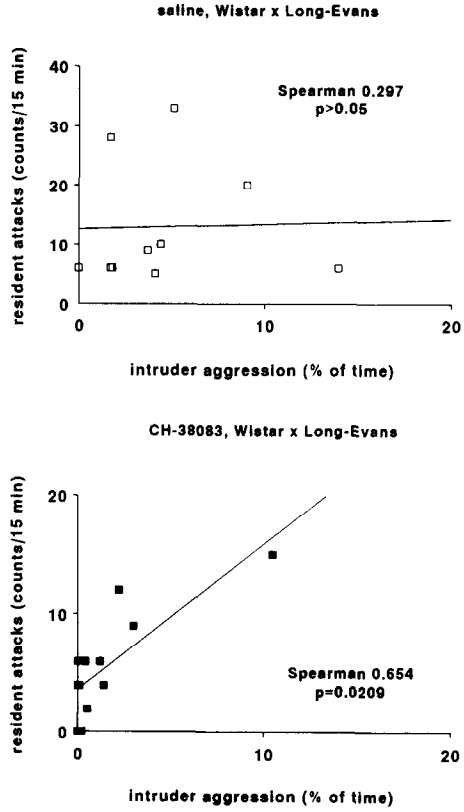

FIG. 2. Correlations between the aggressiveness of the intruders and that of the resident (treated) rats in the W × W saline and W × W CH-38083 groups.

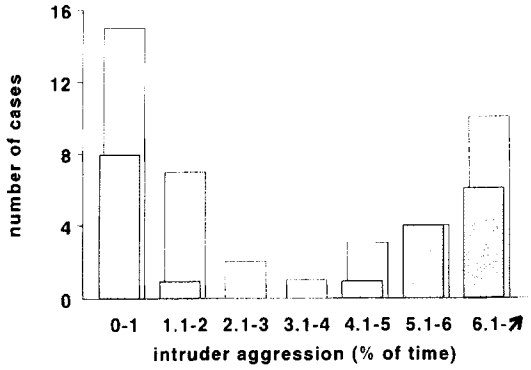

FIG. 3. Histogram showing the frequency distribution of intruders in relation to their aggressiveness without considering the strain to which they belong. Dotted columns indicate the proportion of Wistar rats.

DISCUSSION

In the present experiment there was no general increase of aggressiveness in animals treated with the α_2-adrenoceptor antagonist. The CH-38083, however, induced a significant correlation between the behavior of the intruder and the frequency of biting attacks performed by the treated animals. A similar correlation in saline-treated controls did not occur. Rats with the blocked α_2 adrenoceptors were significantly less aggressive when facing peaceful intruders compared to controls, whereas treated rats encountering aggressive intruders were significantly more aggressive than their saline-treated counterparts. The effect of the treatment seemed to be restricted to biting attacks, because other agonistic behaviors and behaviors not related to aggression (social interactions, exploration) were not affected.

In previous experiments the α_2-adrenoceptor blocker CH-38083 (as well as idazoxan and yohimbine) repeatedly and potently increased the aggressiveness of Long–Evans rats (13). This did not occur in this experiment, which might indicate that α_2 receptor blockade induces aggressiveness in Long-Evans rats while making Wistar rats more responsive to the aggressive behavior of the opponent. In a parallel experiment, however, Long-Evans rats treated with CH-38083 gave (in addition to an increased aggressiveness) a similarly adapted

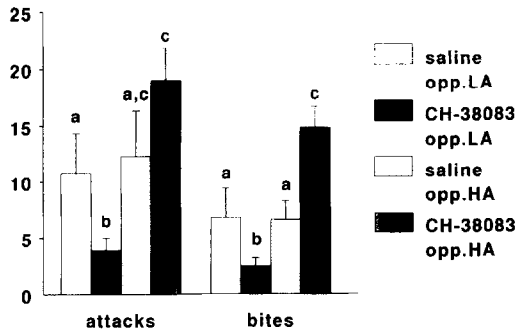

FIG. 4. The frequency of biting attacks and bites in saline- and CH-38083-treated rats grouped according to the aggressiveness of the opponent. opp. LA = faced with low-aggression opponents; opp. HA = faced with high-aggression opponents. Columns labelled with different letters are statistically different ($p < 0.05$).

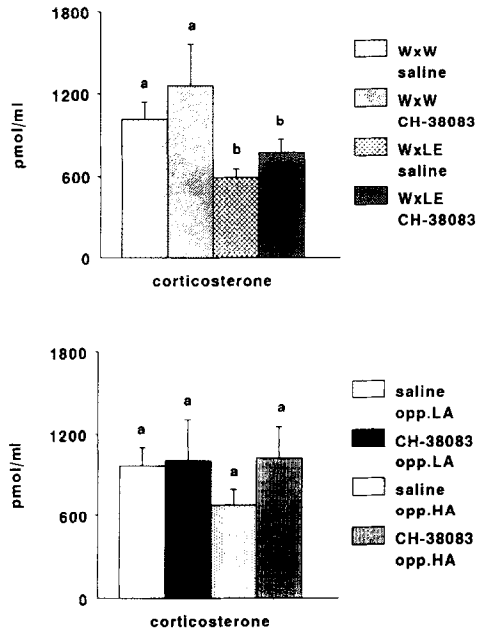

FIG. 5. The results of plasma corticosterone measurements in resident Wistar (W) rats grouped according to the strain of the intruder (W = Wistar; LE = Long-Evans) (top) and according to the aggressiveness of the intruder (bottom), respectively. opp. LA = faced with low-aggression opponents; opp. HA = faced with high-aggression opponents. Columns labelled with different letters are statistically different ($p < 0.05$).

response to the challenge received from the intruder without this occurring in saline-treated animals (12). This might indicate that α_2-adrenoceptor blockade induces a more adapted response to the behavior of the opponent in both Long-Evans and Wistar rats, whereas a general aggression-elevating effect occurs only in the former strain. This could be in correlation with the lower level of aggressiveness of Long-Evans rats (which, thus, might be more prone to elevating effects).

In previous experiments, aggression triggered a significant increase in plasma corticosterone (13). This finding correlated well with the results of others (15,26). Very high corticosterone titers were found in the fighting animals of this experiment. Although the corticosterone response was similar in residents fighting with intruders showing high or low levels of aggressiveness, there was a significant difference between animals fighting with Long-Evans and Wistar rats. Because the main behavioral difference between the two strains was the exaggerated high score of defensive behaviors in Long-Evans rats, one could hypothesize that intruders more engaged in defensive (mainly immobility) behaviors induce a decreased stress response in residents. At the same time the aggressiveness of the intruders seemed to have no effect on the stress response of the resident.

In Long-Evans rats the CH-38083 treatment, which also increased aggressiveness, caused an additional increase in plasma glucocorticoids (13). Whether this increase was due to the increase in the animals' aggressiveness or to the catecholaminergic activation is hard to evaluate, because intracerebroventricular or intraperitoneal administration of catecholamines, as well as the administration of α_2-adrenoceptor antagonists, results in an increase of plasma ACTH and glucocorticoids (4,7,18). No statistically significant increase in

plasma corticosterone was induced by the treatment in the present experiment, although slight increases were obtained in rats fighting with both Wistar and Long-Evans intruders.

Presynaptic α_2 adrenoceptors are involved in the negative feedback control of catecholamine release (3,6). α_2-Adrenoceptor antagonists like yohimbine, idazoxan, and CH-38083 result in an increased norepinephrine release in both the brain and periphery (14,22,23,27). Thus, one would hypothesize that their behavioral actions may be attributed to an emphasized catecholaminergic activation. However, the possibility cannot be ruled out that postsynaptic α_2 adrenoceptors are also involved in the noticed effects.

There are little data on the actions of these agents on aggression. Yohimbine has been shown to inhibit offensive aggression in mice (16), but it caused a significant increase in aggression in rats with the same dosage (9). Our previous findings indicate that at least three of the commonly used α_2-adrenoceptor blockers (yohimbine, idazoxan, and CH-38083) stimulate aggression in rats, although not within the same dose range (13). The present findings support these observations, but only in animals facing highly aggressive opponents. The observation that the behavioral effects of catacholaminergic activation may depend on the behavior of the opponent may explain the discrepancies existing in the literature concerning the role of catecholamines in aggression [see the review by Bell and Hepper (1)]. Because the behavior of the opponent is rarely monitored, it may be that the causes of the contrasting results were hidden in the different degrees of challenge to which the experimental animals had been submitted.

There are only a few reports in which a correlation has been found between behavioral reactivity and the catecholaminergic system. It was shown that depleted sympathetic catecholamine levels induce a deficit of behavioral initiation in active avoidance tests (2). This might mean that one of the behavioral actions of catecholamines is the regulation of behavioral reactivity to environmental challenges (10). Because it was also shown that the α_2-adrenoceptor agonist medetomidine may exert a stress protective effect (24), it might be assumed that not only behavioral but also physiological reactivity to challenges might be regulated by α_2 adrenoceptors.

The main finding of our experiment is that rats treated with α_2-adrenoceptor antagonists show an aggressive response that is more adapted to the behavioral challenge received from the opponent compared with saline-treated controls. Both between-group differences and within-group individual differences support this conclusion. This effect may indicate that catecholamines affect behavior and aggressiveness in a previously unrecognised way: they change the reactiveness of animals to the behavioral actions of the opponent.

ACKNOWLEDGEMENTS

The author is grateful to Hupcsik Kornélia for her technical assistance. This work was supported by OTKA Grants No. F 5099 and T 012932.

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