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Strain Differences in Clonidine-Induced Aggressiveness in Mice and its Interaction With the Dopamine System

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NIKULINA, E. M. AND V. KLIMEK. *Strain differences in clonidine-induced aggressiveness in mice and its interaction with the dopamine system.* PHARMACOL BIOCHEM BEHAV **44**(4) 821-825, 1993.—The influence of a genotype of inbred mice on the aggressive behavior induced by clonidine and the role of dopamine D_1 and D_2 receptors in that behavior were studied. Clonidine in a dose of 10 mg/kg evoked a strong aggressiveness in BALB/c, DBA/1, and CC57Br mice and an intermediate response in C57BL/6J, Albino Swiss, and CBA mice, whereas DD, A/He, and C3HA/y mice did not show any aggressive behavior. Apomorphine significantly potentiated the clonidine-induced aggressiveness in C57BL/6J mice. In Albino Swiss mice, SK&F38393 as well as quinpirole augmented the aggressive behavior evoked by clonidine. The clonidine-induced aggressiveness was blocked by SCH23390 and *cis*-flupentixol but not by (−)-sulpiride. In aggressive mice, the binding of [^{3}H]SCH23390 was decreased in the limbic forebrain, whereas the binding of [^{3}H]spiperone was not changed. The obtained results indicate that the potency of the clonidine-induced aggressiveness depends upon genotype of mice; moreover, the presence of a physiological function of D_1 receptors is necessary for its occurrence.

Clonidine Aggression Genotype Dopamine receptors

CLONIDINE, an agonist of α-adrenoceptors, given at a high dose induces aggressiveness in mice, similar to the competitive or intermale aggression observed in male animals of the same species when a social rank of choice is in dispute (fighting, biting) according to Valzelli (25). The clonidine-induced aggressiveness has been practically demonstrated in one strain of mice (13,16). A number of inbred strains of mice that are genetically homogenous are known for certain behavioral patterns, ranging from various levels of the spontaneous activity to diverse responses to apomorphine and amphetamine (5,26) as well as clonidine administration (8).

It was previously demonstrated that the clonidine-induced aggressiveness was presumably caused by α_1-adrenergic stimulation (12,13). The same authors showed the clonidine-induced aggressiveness to be inhibited by some neuroleptics, for example, spiperone or *cis*-flupentixol; thus, an assumption is put forward that dopaminergic transmission is necessary for the occurrence of clonidine-induced aggressiveness. However, clonidine interaction with the dopaminergic system has not been studied as yet in this model of response. A possibility of a functional interaction between the clonidine-induced stimulation of α-adrenergic receptors and dopaminergic receptor subtypes has arisen from the recent use of highly selective D_1 and D_2 dopamine receptor agonists and antagonists (2). Further, Tassin et al. (21) suggested that noradrenergic fibers may contribute to regulation of the D_1 receptor sensitivity.

The aim of the present article was to investigate the influence of a genotype of inbred mice on the clonidine-induced aggressiveness, as well as the role of dopamine (D_1 and D_2) receptors in this behavior.

METHOD

Animals

The experiments were carried out on males of inbred strains of mice: BALB/c, C57BL/6J, CBA, DD, A/He, CC57Br, DBA/1, and Albino Swiss, weighing 22-35 g at the age of 3-4 months, housed in groups (eight animals per cage), and having free access to food and water. Animals were kept under natural daylight conditions; tests were performed between 10:00 a.m. and 1:00 p.m. in the light room. All mice were used only once. In all of four mice observed together, each received the drug treatment.

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Drugs

The following drugs were used: clonidine HCl (Boehringer-Ingelheim, Ridgefield, CT), apomorphine HCl (Sigma Chemical Co., St. Louis, MO), *cis*-flupentixol (Lundbeck, Copenhagen, Denmark), haloperidol (Gedeon Richter), SCH23390 (Schering, Kenilworth, NJ), SK&F38393 (Smith, Kline and French, Philadelphia, PA), quinpirole HCl (Lilly, Indianapolis, IN), and (−)-sulpiride (Sigma). Drugs were dissolved in distilled water and used in a volume of 10 ml/kg. All drugs except apomorphine were injected IP; apomorphine was administered SC. Control mice were injected with distilled water.

Behavioral Experiments

Aggressive behavior was induced by clonidine given in a dose of 10 mg/kg; in some experiments, the doses were increased to 25 and 50 mg/kg. The aggressiveness was assessed by counting the number of biting attacks within 1 h after clonidine administration. Immediately after clonidine injection, groups of four mice of equal weight from the same home cage were placed together in glass cylinders and observed for 1 h. In the experiments in which dopamine agonists and antagonists were applied, apomorphine, SK&F38393 and quinpirole were given simultaneously with clonidine; flupentixol, haloperidol, sulpiride, and SCH23390 were administered 15 min before clonidine.

The obtained data were statistically analyzed by a two-factor analysis of variance (ANOVA), the factors being strain and treatment (clonidine). An analysis of individual between-group comparisons was carried out using Student's *t*-test and Dunnett's procedure.

Binding Assays

For dopamine receptor binding studies, Albino Swiss mice were killed 2.5 h after clonidine or distilled water (control) injections. The tissue, striatum, or limbic forebrain (containing the olfactory tubercle, preoptic area, nucleus accumbens, septum, amygdala, and ventral cortex) was dissected out, frozen, and pooled from three mice for one estimation.

The tissue was homogenized in 20 vol (w/v) ice-cold potassium phosphate buffer (50 mM, pH 7.4) using a Polytron homogenizer for 15 s and centrifuged at 25,000× *g* for 15 min. That step was repeated twice. Final pellets were resuspended in 100 (striatum) or 50 (limbic forebrain) vol (w/v) potassium phosphate buffer (pH 7.4) and assay procedure was carried out according to Maj et al. (14). The radioligand used were: [^{3}H]SCH23390 (specific action 72.2 Ci/mmol; Amersham Corp., Arlington Heights, IL) and [^{3}H]spiperone (specific action 40 Ci/mmol; New England Nuclear, Newton, MA) for labeling D_1 and D_2 receptors, respectively.

Six increasing concentrations of [^{3}H]SCH23390, ranging from 0.05–2.0 nM, and of [^{3}H]spiperone, ranging from 0.03–1.0 nM, respectively, were used for equilibrium saturation experiments. The B_{max} and K_d values were calculated by the Scatchard analysis, and the points were assayed in duplicates. The D_1 nonspecific binding was defined in the presence of 1 μM *cis*-flupentixol and the D_2 nonspecific one in the presence of 1 μM (+)-butaclamol.

Analysis of variance, followed by a protected lowest significant difference test, was applied to determine the statistical significance of the obtained results.

RESULTS

Interstrain Differences in the Clonidine-induced Aggression

Acute administration of clonidine (10 mg/kg) induced aggressive behavior in several strains of mice. Injection of distilled water into intact mice did not produce any aggression in the used strains of mice. The average intensity of the clonidine-induced aggression was 40 ± 5.8 attacks per hour, close to the mean number of attacks described previously for the Albino Swiss strain of mice (12,13); however, the intensity of clonidine-induced aggression was different in various strains of mice. The most pronounced effect was seen in BALB/c, DBA/1, and CC57Br mice; they fought during the whole session (Fig. 1). An intermediate response was found in C57BL/6J, Albino Swiss, and CBA mice. DD and A/He mice did not reveal any biting attacks. Two-way ANOVA (one factor = strain, one factor = treatment) used for statistical assessment showed a significant interaction between the two factors, indicating that the effect of clonidine is genotype dependent, $F(8, 46) = 29.1$, $p < 0.001$. The variability between strains is significantly higher than interstrain one.

Administration of higher doses of clonidine (25 and 50 mg/kg) to weakly responding A/He mice evoked some symptoms of aggressive behavior; however, the intensity of aggression was not stronger. Another strain (the DD) showed the clonidine-induced aggression only after a dose of 50 mg/kg (Table 1), yet the number of biting attacks was five times smaller than in aggressive strains of BALB/c and DBA/1 mice (the clonidine dose = 10 mg/kg).

Influence of the Dopamine System on the Clonidine-induced Aggression

Different strains of mice were selected to study the effect of the dopamine system on the clonidine-induced aggression on strongly (CC57Br), intermediately (C57BL/6J and Albino Swiss), and weakly responding (DD) mice.

The dopamine agonist apomorphine, given in a dose of 1 mg/kg, potentiated the clonidine-induced aggression in C57BL/6J mice, as well as in DD ones. The selective agonists of dopamine D_1 and D_2 receptors, SK&F38393 and quinpirole, respectively, potentiated in a dose-dependent manner the clonidine-induced aggressiveness in Albino Swiss mice (Table 2).

The effect of dopamine antagonists on the clonidine-induced aggressiveness is shown in Table 3. SCH23390, a specific antagonist of D_1 receptors, inhibited the aggressiveness of CC57Br as well as Albino Swiss mice. The mixed antagonist of both D_1 and D_2 receptors *cis*-flupentixol decreased the aggressive behavior, yet to a lesser extent than did SCH23390. Interestingly, (−)-sulpiride, the specific D_2 receptor antagonist, did not influence the clonidine-induced aggression in Albino Swiss mice.

Binding Studies

The clonidine-induced aggressiveness led to a decrease by ca 21% in the density (B_{max}) of dopamine D_1 receptors, labeled with [^{3}H]SCH23390, in the limbic forebrain of Albino Swiss mice. Neither the number of D_1 binding sites in the striatum nor the density of D_2 receptors in either brain region were changed (Table 4).

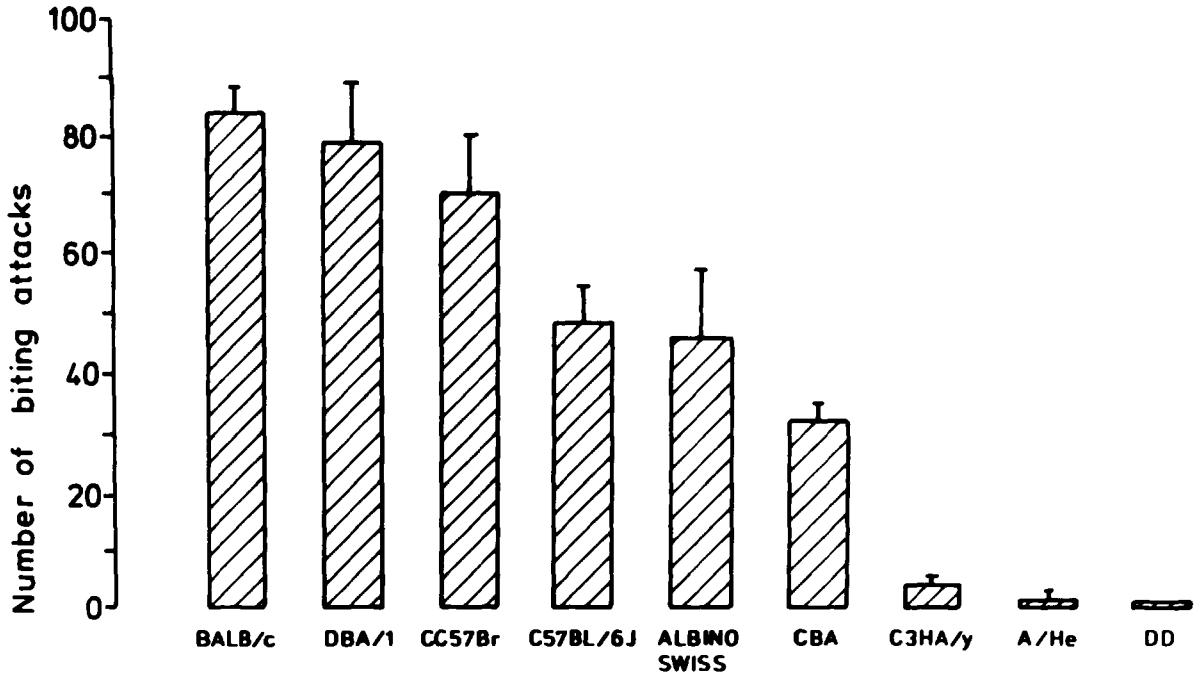

FIG. 1. Clonidine-induced aggressiveness in different strains of mice. The number of biting attacks was assessed within 1 h beginning immediately after injection of clonidine in a dose of 10 mg/kg. Data represent mean ± SEM from six to seven trials.

DISCUSSION

Our present results indicate profound differences in the reactivity of various strains of mice to clonidine administration, which evokes aggressive behavior. BALB/c, DBA/1, and CC57Br mice strongly reacted to the clonidine-induced aggressiveness; animals made biting attacks during the whole session. In contrast, DD and A/He mice did not show any aggressive reactions after administration of the same dose of clonidine; however, in the case of further increased doses of the compound some aggressiveness was observed. Other strains of mice, C57BL/6J, Albino Swiss, and CBA, showed a moderate aggressiveness evoked by clonidine. It is noteworthy that other authors reported different reactions to the clonidine- and amphetamine-induced locomotor activity in CD-1 and C57BL/6J mice, which suggests a lower or higher sensitivity, respectively, of central adrenoceptors (8). Different responses to apomorphine and amphetamine administration to various strains of mice have also been reported (5,11,26,27). Maj et al. (13) suggested that clonidine given in high doses induces aggressiveness during stimulation of α_1-adrenoceptors in mice. Therefore, it may be concluded that there exists a genotype-dependent, different sensitivity of α_1-adrenoceptors in various strains of mice. The strain-dependent difference in brain adrenergic neurons and their relation to α_1-adrenoceptors density was demonstrated by Vantini et al. (24). The basic significance of the genotype-dependent functional differences found in the catecholaminergic system consists of the activity of the key enzyme tyrosine hydroxylase. A decreased activity of tyrosine hydroxylase was found in CBA mice in comparison with BALB/c ones (3). The activity of that enzyme was higher in BALB/c mice than in DBA/1 or C57BL/6J ones (22). In our experiments, BALB/c and DBA/1 mice showed similar potency in responding to the clonidine-induced stimulation of aggression; therefore, the tyrosine hydroxylase activity is not well correlated with the intensity of these response.

As was demonstrated by Maj et al. (13), the clonidine-induced aggressive behavior was inhibited by several neuroleptics, which can suggest that the presence of dopaminergic transmission is necessary for the occurrence of aggressiveness. Based upon these observations, one could expect that combined administration of clonidine and dopaminergic agonist results in enhancement of aggressive behavior. The existence of such interaction between dopamine- and noradrenaline-containing neurons in the CNS was described by several authors (1,4,7,18).

Our results seem to support this assumption showing that apomorphine, an agonist of both dopamine D_1 and D_2 recep-

TABLE 1

AGGRESSIVENESS INDUCED BY VARIOUS DOSES OF CLONIDINE IN DD AND A/He MICE

Strain of Mice	Dose of Clonidine (mg/kg)	*n*	Number of Biting Attacks Within 1 h (mean ± SEM)
DD	10	6	0
	25	6	0
	50	5	14.0 ± 5.5
A/He	10	6	2.0 ± 0.6
	25	6	13.5 ± 3.7*
	50	5	8.8 ± 1.9*

*$p < 0.05$.
n, number of trials.

TABLE 2

EFFECTS OF DOPAMINERGIC AGONISTS ON CLONIDINE (10 mg/kg)-INDUCED AGGRESSION IN VARIOUS STRAINS OF MICE

Strain of Mice	Treatment	Dose (mg/kg)	n	Number of Biting Attacks Within 1 h (mean ± SEM)
C57BL/6	Vehicle		6	48.3 ± 3.4
	Apomorphine	1.0	6	82.3 ± 10.4*
DD	Vehicle		6	0
	Apomorphine	1.0	6	20.3 ± 3.2
	Apomorphine	2.5	6	38.1 ± 4.4
Albino Swiss	Vehicle		14	47.5 ± 8.8
	SK&F38393	5.0	6	87.8 ± 17.6
	SK&F38393	10	6	119.5 ± 20.0†
	Quinpirole	0.1	6	58.7 ± 4.1
	Quinpirole	1.0	6	146.0 ± 19.5†

Compounds were injected simultaneously with clonidine. n, number of trials.
*$p < 0.05$, †$p < 0.001$ (compared to vehicle-injected group).

TABLE 3

EFFECTS OF DOPAMINERGIC ANTAGONISTS ON CLONIDINE (10 mg/kg)-INDUCED AGGRESSION IN DIFFERENT STRAINS OF MICE

Strain of Mice	Treatment	Dose (mg/kg)	n	Number of Biting Attacks Within 1 h (mean ± SEM)
CC57Br	Vehicle		6	76.6 ± 12.8
	SCH23390	0.2	5	8.6 ± 3.1*
	cis-Flupentixol	0.1	6	22.0 ± 4.5†
	Haloperidol	0.1	6	0.0 ± 0.0
Albino Swiss	Vehicle		10	48.8 ± 10.4
	SCH23390	0.15	6	8.0 ± 2.9†
	(−)-Sulpiride	10.0	6	43.2 ± 9.0

n, number of trials. Compounds were injected 15 min before clonidine.
*$p < 0.01$, †$p < 0.05$.

TABLE 4

EFFECT OF CLONIDINE (10 mg/kg)-INDUCED AGGRESSIVENESS OF BINDING OF [^{3}H]SCH23390 AND [^{3}H]SPIPERONE IN THE ALBINO SWISS MICE STRIATUM (A) AND LIMBIC FOREBRAIN (B) HOMOGENATES

	[^{3}H]SCH23390		[^{3}H]Spiperone	
Treatment	B_{max} (pmol/g)	K_d (nM)	B_{max} (pmol/g)	K_d (nM)
A				
Vehicle	64.25 ± 3.91	0.32 ± 0.02	29.75 ± 2.21	0.16 ± 0.02
Clonidine	60.95 ± 3.20	0.29 ± 0.03	28.75 ± 2.01	0.10 ± 0.01
B				
Vehicle	21.13 ± 1.14	0.40 ± 0.04	9.55 ± 1.01	0.12 ± 0.01
Clonidine	16.80 ± 1.32*	0.34 ± 0.03	9.25 ± 0.98	0.13 ± 0.01

The number of biting sites, B_{max}, and binding affinity constants, K_d values, were calculated by Scatchard analysis; the mean values of five to six separate determinations and SEM are given.
*$p < 0.05$.

tors (6,10,20,23), potentiates the clonidine-induced aggressiveness in C57BL/6J and DD mice. The selective agonists of both D_1 and D_2 dopamine receptors SK&F38393 and quinpirole (2), respectively, potentiated the aggressive behavior evoked by clonidine; this finding points to involvement of both dopamine receptors in this type of aggression. On the other hand, the selective antagonist of dopamine D_1 receptors, SCH23390, as well as *cis*-flupentixol, which blocks both D_1 and D_2 receptors (9), significantly attenuated the clonidine-induced aggressiveness. Haloperidol, a neuroleptic with a wide spectrum of activity that also blocks both dopamine receptors, totally abolished that behavior. However, (−)-sulpiride, a selective D_2 antagonist (15), did not affect the clonidine-induced aggression. It is likely that the dose used was too low to block efficiently D_2 receptors or the endogenous dopaminergic tone into D_1 receptors is sufficient and, at the same time, necessary for the expression of the clonidine-induced aggressiveness. It seems worthy to note that D_1 receptors that are localized postsynaptically correlate well with the distribution of dopaminergic afferents (19).

Binding studies seem to confirm our suggestion, as they show a decreased density of D_1 receptors after clonidine administration, which probably resulted from a dynamic stimulation of those receptors by endogenous dopamine. The fact that the density of D_1 receptors was decreased in the limbic forebrain may help localize the region responsible for the clonidine-induced aggressiveness. The above results are consistent with the earlier findings of Pucilowski et al. (17), who studied aggressiveness after various lesions of the CNS and showed the limbic forebrain to be a region of the utmost importance.

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