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Stimulation of Beta-Adrenergic Receptors and Spontaneous Motor Activity in Mice

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GOLDSCHMIDT, P. L., H. FRANCES AND P. SIMON. *Stimulation of beta-adrenergic receptors and spontaneous motor activity in mice.* PHARMACOL BIOCHEM BEHAV 21(2) 177–180, 1984.—The effects of 3 beta-adrenergic agonists (clenbuterol, isoproterenol and salbutamol) on the spontaneous motor activity of mice were studied. The present research indicated that motor activity was significantly decreased 30 minutes after IP injection of either clenbuterol (0.06 mg/kg), isoproterenol (0.5 mg/kg) or salbutamol (2 mg/kg). Hypomotility induced by clenbuterol was also significantly antagonized by propranolol in doses ranging from 1 to 8 mg/kg and by penbutolol in doses from 0.03 to 0.5 mg/kg. However, practolol, which does not cross the blood brain barrier, did not antagonize the effect of clenbuterol. Therefore, it may be hypothesized that beta adrenergic agonists decrease motor activity by a central mechanism. It was also found that tachyphylaxis or resistance to treatment, observed in cardiovascular and bronchopulmonary systems with beta-adrenergic agonists, developed after 7 injections of clenbuterol (0.25 mg/kg IP, twice daily) in the behavioral model of spontaneous motor activity in mice.

Beta-receptor agonists Clenbuterol Desensitization Isoproterenol Mice Motor-activity
Penbutolol Practolol Propranolol Tachyphylaxis

THE activity profile of beta-adrenergic agonists in animal studies suggests that they may act as antidepressants in man [10]. These experimental results were later confirmed clinically [14,15]. Studies also indicate that after prolonged use of beta-adrenergic agonists as bronchodilators, patients become hyposensitive to subsequent treatment [6,18]. Avner and Noland [3] proposed that the desensitization might be due to a change in the affinity of receptors for the ligand. Chuang *et al.* [5] developed the notion that the ligand-receptor complex might be internalized after repeated stimulation with the agonist. According to Reaka and Samuels [17], the decrease in effectiveness might result from variations in receptor "turnover." In intact animals, Conolly *et al.* [7] demonstrated that resistance to the cardiovascular activity of isoproterenol develops after prolonged exposure to the drug. Several authors have also reported changes in the bronchopulmonary response to beta-adrenergic agonists after repeated treatment [1, 2, 18, 19, 20, 21]. In addition, changes in the density of beta-adrenergic receptors and their responsiveness were observed in fragments of rat cortex after pretreatment with beta-adrenergic agonists [8] and a decrease in rat cerebellum beta-adrenergic receptors was reported after peripheral perfusion with clenbuterol [9]. To our knowledge, no data exist giving evidence, in behavioral models, of a reduction in the effect of a test dose (tachyphylaxis) after repeated treatments with beta-adrenergic agonists. As beta-adrenergic agonists provoke hypomotility in mice and rats [10,12], which is not usually observed with antidepressants, the present study was undertaken, using the model of spontaneous motor activity in mice, to determine whether the effect on motility of beta-adrenergic agonists includes central effects. Furthermore we tried to determine whether beta-receptors are involved in the induced hypomotility and whether resistance to treatment ("tachyphylaxis"), observed in bronchopulmonary and cardiovascular systems, could develop in this behavioral model.

METHOD

Animals

The experiments were carried out using naive male Swiss NMRI (Naval Medical Research Institute) mice (20–25 g) from CERJ (Centre d'Elevage Roger Janvier, France), housed in groups of 10 under standard conditions (light/dark cycle = 12 hr/12 hr; room temperature = 21 ± 1°C) with free access to food and water.

Substances

We studied 3 beta-adrenergic agonists: Clenbuterol (Boehringer Ingelheim), Isoproterenol (Aldrich Europe), Salbutamol (Glaxo), and 2 beta-adrenergic antagonists: Penbutolol (Hoechst), Practolol (ICI Pharma).

Treatment

All drugs were administered intraperitoneally, in water solution, in a volume of 0.25 ml per 20 g body weight. Control mice received the same volume of vehicle at the same

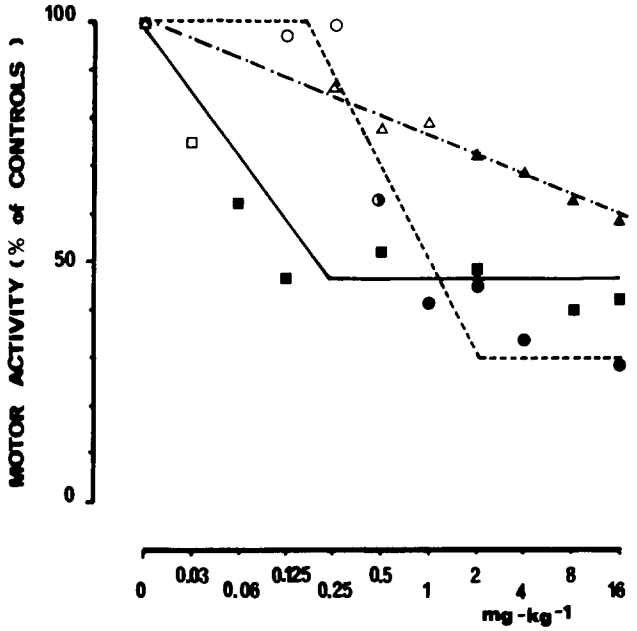

FIG. 1. Effect of isoproterenol, salbutamol and clenbuterol on spontaneous motor activity in mice. Drugs were administered as indicated in the Method section. Half-black symbols indicate statistically significant difference $p<0.05$, black symbols indicate $p<0.01$. Ten animals were used for each dose. ○— — —○ isoproterenol, △—·—△ salbutamol, □—□ clenbuterol. Absolute value for controls: isoproterenol: 402±29; salbutamol 390±31 and clenbuterol 415±38.

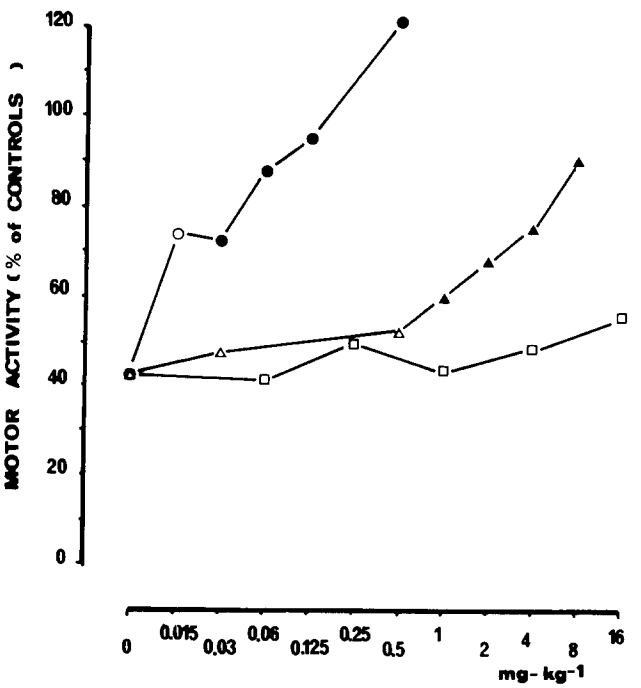

FIG. 2. Antagonism of clenbuterol-induced hypomotility by propranolol, penbutolol, and practolol. The antagonists were administered to groups of 10 animals per dose, 15 minutes before administration of clenbuterol (0.5 mg/kg) and 45 minutes before the start of the test. Black symbols indicate a statistical difference $p<0.01$. ○—○ penbutolol, △—△ propranolol, □—□ practolol. Absolute value for controls in the experience with penbutolol; 291±30; with propranolol 337±20 and with practolol 260±26.

time and by the same route. Ten animals were used for each dose of each substance.

Determination of the Effects of Beta-Adrenergic Agonists on Motor Activity in Mice

The activity of each mouse was determined in a photocell counter [4] consisting of translucent boxes (26×21×10 cm) with two photocells mounted at a 90° angle 1 cm above the box floor. The mice were placed in the individual cages of the actimeter after IP administration of the drugs (see tables for time indications). Activity was reported as the sum of the interruptions of each photocell light beam recorded for 30 minutes, immediately after placing the mice in the test box.

Study of the Effects of Beta-Adrenergic Antagonists (Penbutolol, Propranolol and Practolol) on the Clenbuterol-Induced Hypomotility

Beta-adrenergic antagonists were administered 45 minutes before placing the mice in the test box and 15 minutes before administration of beta-adrenergic agonist. Motor activity was recorded for 30 minutes, immediately after placing the mice in the test box.

Detection of Resistance to Treatment With a Beta-Adrenergic Agonist After Repeated Administration of the Same Substance ("Tachyphylaxis")

Groups of 10 animals were pretreated with either 3 or 7 injections of clenbuterol (0.25 mg/kg IP) or with the solvent, according to the following schedule: first administration between 8.30 and 9.30 a.m.; second administration between 5.30 and 6.30 p.m.; third administration between 8.30 and 9.30 a.m. the following day, etc.

TABLE 1

EFFECT OF PROPRANOLOL, PENBUTOLOL (AT DOSES WHICH ANTAGONIZE CLENBUTEROL INDUCED HYPOMOTILITY) AND PRACTOLOL, ON SPONTANEOUS MOTOR ACTIVITY IN MICE

Treatment (45 min before the test)	Light beams crossed during 30 minutes	
distilled water	*100 ± 19	NS
propranolol 1 mg/kg	119 ± 18	
propranolol 8 mg/kg	77 ± 15	
distilled water	†100 ± 13	NS
penbutolol 0.06 mg/kg	118 ± 20	
penbutolol 8 mg/kg	116 ± 12	
distilled water	‡100 ± 11	NS
practolol 8mg/kg	93 ± 10	
practolol 16 mg/kg	125 ± 12	

Ten animals were used for each dose.

Absolute values for control groups: *=268 ± 50; †=218 ± 29; ‡=309 ± 35.

TABLE 2

EFFECTS OF 0.125 AND 0.5 mg/kg OF CLENBUTEROL ADMINISTERED IP 30 MIN BEFORE THE ANIMALS WERE PLACED IN THE ACTIMETER, AND 6 HOURS AFTER THE LAST OF 3 OR 7 PRETREATMENTS WITH CLENBUTEROL (0.25 mg/kg) OR WATER ON SPONTANEOUS ACTIVITY IN MICE

Pretreatment	3 administrations (*)		7 administrations (†)	
	Light Beams Crossed During 30 Minutes (% of control)			
Treatment	water	clenbuterol	water	clenbuterol
Clenbuterol 0.125 mg/kg	45 ± 6	51 ± 6	52 ± 7 $p<0.001$	84 ± 8
Clenbuterol 0.5 mg/kg	37 ± 6	38 ± 4	54 ± 6 $p<0.05$	86 ± 14

*Absolute value for control animals pretreated 3 times with distilled water and treated with distilled water: 429 ± 44.

†Absolute value for control animals pretreated 7 times with distilled water and treated with distilled water: 368 ± 60.

Ten animals were used for each dose.

Resistance to the effect of clenbuterol was tested with two doses of the drug (0.125 and 0.5 mg/kg) known to cause hypomotility (see Fig. 1), administered to the pretreated animals (3 or 7 administrations) 30 minutes before they were placed in the test box. Motor activity was recorded for 30 minutes, immediately after placing the mouse in the test box.

Statistical Analysis

The mean number of light beams crossed by each group of treated mice was compared to control values using Student's *t*-test. In the figures and in the tables the results of each are represented as the % of controls.

RESULTS

Figure 1 shows that the three beta-adrenergic agonists studied reduced spontaneous motor activity in mice. Clenbuterol significantly ($p<0.01$) reduced motor activity at a dose of 0.06 mg/kg. The dose of isoproterenol required was almost 10 times higher and that of salbuamol 40 times higher.

To determine whether the hypomotility induced by beta-adrenergic agonists could be prevented by pretreating the animals with a beta adrenoreceptor blocker, propranolol, penbutolol and practolol were administered 15 minutes before clenbuterol, at a dose which falls in the plateau of the hypomotility dose-response curve (0.5 mg/kg). Figure 2 shows that propranolol, starting at a dose of 1 mg/kg, was able to antagonize significantly the hypomotility induced by clenbuterol. The effect was dose dependent between 1 and 8 mg/kg and was almost completely suppressed by 8 mg/kg. Penbutolol significantly antagonized clenbuterol-induced hypomotility starting at 0.03 mg/kg and the effect was almost total between 0.06 and 8 mg/kg. The value observed with 0.5 mg/kg of penbutolol (122%) was higher than controls, but not significantly different. Practolol, in doses ranging from 0.06 to 16 mg/kg, was not able to antagonize the effect of clenbuterol. Neither penbutolol nor propranolol affected motor activity when administered alone at doses which antagonized agonist-induced hypomotility and practolol even at 8 to 16 mg/kg did not significantly affect motor activity of mice (Table 1).

Table 2 shows that 3 administrations of clenbuterol at a dose of 0.25 mg/kg were not able to induce resistance to treatment with the same drug, at half or double the dose. There were no significant differences in treatment either with 0.125 or 0.5 mg/kg of clenbuterol or with distilled water. After 7 pretreatments with clenbuterol (0.25 mg/kg), animals developed resistance to subsequent treatment with both 0.125 and 0.5 mg/kg of clenbuterol. Clenbuterol was no longer able to induce hypomotility. The control groups pretreated with clenbuterol and treated with distilled water were not significantly different from controls pretreated with distilled water and treated with distilled water (311±50 and 368±60 respectively), indicating that clenbuterol leaves no residual effects on spontaneous motor activity 6 hours after IP administration. The motility scores for animals pretreated with clenbuterol (0.25 mg/kg) and treated with 0.125 or 0.5 mg/kg of clenbuterol were not significantly different (309±31 and 315±50 respectively) indicating that even the double of the dose used to desensitize animals was not able to induce hypomotility. The absolute value for the control group treated with distilled water after 3 or 7 pretreatments with distilled water was not significantly different (429±44 and 368±60 respectively).

DISCUSSION

Francès *et al.* [10] reported that beta-adrenergic agonists decrease locomotion in mice. It was later shown that the effect of isoproterenol on motor activity can be antagonized by beta-adrenoreceptor blockers [11]. The present results obtained with clenbuterol, a beta-adrenergic agonist which was several times more active than other drugs, confirm these effects. The active doses of clenbuterol causing a significant decrease in motor activity were around 0.06 mg/kg, whereas isoproterenol was active at 0.5 mg/kg, and salbutamol at 2 mg/kg. The hypomotility induced by clenbuterol could be completely antagonized by propranolol and penbutolol, beta-adrenoreceptor blockers which diffuse easily

across the blood brain barrier. However, practolol, which penetrates the central nervous system poorly [13], did not—under our experimental conditions—antagonize the clenbuterol-induced hypomotility. Hence, the hypomotility induced by clenbuterol seems to result from a central effect.

Using repeated administrations to induce resistance to treatment (tachyphylaxis or subsensitivity), it was observed that after 7 intraperitoneal injections of clenbuterol, subsequent administration at either lower or higher doses was no longer able to induce hypomotility. Total resistance, under our experimental conditions, developed only after 7 administrations given over at least 3 successive days. This effect could not be obtained after 3 administrations. The results presented here, showing agonist-induced desensitization in the model of spontaneous motor activity in mice are in agreement with those of Przegalinski *et al.* [16] who reported that pretreating rats for 4 days with antidepressants which block noradrenaline uptake, prevents salbutamol-induced hypomotility in the "open-field" test.

Dooley and Hauser [9] reported that peripheral infusion of clenbuterol induced a decrease in the number of beta adrenergic receptors in the rat cerebellum, and Zsilla *et al.* [22] showed that deprenyl, which inhibits rather specifically the degradation of noradrenaline, causes a decrease in the number of beta-adrenergic receptor sites and adenylate cyclase activity in mouse brain.

Since pretreatment with the stimulatory agent decreases or prevents the appearance of hypomotility, it can be hypothesized that the agonist interacts with specific biological structures such as receptors, the repeated stimulation of which induces a reduction in their number.

In view of these results, it would be useful to develop new behavioral tests with which to analyse the role of beta-adrenergic receptors in the CNS, particularly with respect to the biological determinants of depression and/or its treatment.

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