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Peripheral Mediation of Effects of Clenbuterol on Locomotor and Investigatory Behavior in Rats

MARK A. GEYER[1] AND SARAH F. FRAMPTON

Department of Psychiatry, School of Medicine
University of California, San Diego, La Jolla, CA 92093

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GEYER, M. A. AND S. F. FRAMPTON. *Peripheral mediation of effects of clenbuterol on locomotor and investigatory behavior in rats.* PHARMACOL BIOCHEM BEHAV 30(2) 417–420, 1988.—Clenbuterol is one of the few beta adrenergic agonists that readily passes the blood-brain barrier. Hence, the behavioral effects in rats of systemic administrations of clenbuterol have been used as a reflection of the activation of central beta receptors. The present experiments were designed to test the hypothesis that the reduction in locomotor activity induced by clenbuterol is mediated by central rather than peripheral beta receptors. First, dose-dependent reductions in ambulation, holepoking, and rearing were established following intraperitoneal injections of 0.004 to 1.0 mg/kg clenbuterol. These effects were then found to be similar to those of 0.4 mg/kg isoproterenol, a mixed beta adrenergic agonist that does not enter the brain after systemic administration. The behaviorally suppressive effects of either 0.4 mg/kg isoproterenol or 0.05 mg/kg clenbuterol were found to be completely antagonized by pretreatment with a 10.0 mg/kg dose of nadolol, a beta antagonist that does not penetrate the brain when administered systemically. Nadolol itself had no significant effects on behavior. These results indicate that these behavioral effects of systemic administrations of clenbuterol are mediated by the activation of peripheral rather than central beta adrenergic receptors.

Rats Locomotor activity Investigatory behavior Holeboard Clenbuterol Nadolol Isoproterenol Beta receptors

LARGELY because of the apparent down-regulation of central beta adrenergic receptors by antidepressant drugs [17], considerable interest has been generated in the behavioral and potentially therapeutic effects of centrally acting beta agonists. There is much evidence that clenbuterol, a beta adrenergic agonist which passes the blood-brain barrier, down-regulates some central adrenergic receptors and decreases locomotor activity in rodents [3, 9, 13, 14]. Clenbuterol is highly lipophilic and almost certainly passes the blood-brain barrier [9]. It has been suggested that the decrease in locomotor activity induced by systemic administrations of clenbuterol is a result of its agonist action at beta receptors within the central nervous system [8,14]. This conclusion was based primarily on the observation that practolol, a peripheral beta adrenergic antagonist, was unable to block behavioral effects of clenbuterol [8,16]. However, practolol is primarily a beta-1 antagonist [11] and therefore would not be expected to block the peripheral beta-2 receptors affected by clenbuterol. Recent work has indicated that the sedative effects of clenbuterol in rodents is attributable to beta-2 receptor activation [4]. By contrast, there is as yet no substantial evidence that the clenbuterol-induced decrease in locomotor activity is a central as opposed to a peripheral effect.

The present study was undertaken in order to systematically differentiate between the central and peripheral effects of clenbuterol on the investigatory and locomotor behavior of rats. Two experimental approaches were used to test the general hypothesis that the sedative effects elicited in rats by clenbuterol are centrally mediated. The first approach was to see if isoproterenol, a mixed beta agonist which does not pass the blood-brain barrier [7], would have the same effects in rats as does clenbuterol. The second approach was to see if the effects of clenbuterol on locomotor activity could be blocked by nadolol, a beta adrenergic antagonist which does not penetrate the brain appreciably because of its low lipid solubility [10]. In addition, to confirm the effectiveness of the given dose of nadolol, its ability to block the behavioral effects of isoproterenol was tested.

METHOD

Animals

The animals were experimentally naive male Sprague-Dawley rats weighing 275–300 g. Upon receipt from the supplier (Batton and Kingman), the rats were housed in pairs in a temperature-regulated (25±2°C) animal room on a 12/12 light/dark cycle with free access to food and water. Each

[1]Requests for reprints should be addressed to Mark A. Geyer, Ph.D., Department of Psychiatry, T-004, University of California, San Diego, La Jolla, CA 92093.

group was allowed a seven day period for acclimation to the animal room before behavioral testing, during which time the animals were handled daily.

Drugs

Clenbuterol HCl (Dr. Karl Thomas, GMBH Biberachanderriss) was dissolved in saline at concentrations of 0.004, 0.01, 0.025, 0.05, 0.1, 0.5, and 1.0 mg/ml. L-Isoproterenol HCl (Sigma) was dissolved in saline at a concentration of 0.4 mg/ml. Nadolol (Squibb) was dissolved at a concentration of 10 mg/ml in a vehicle consisting of distilled water with HCl at a pH of 3. All drugs were administered intraperitoneally in a volume of 0.1 ml per 100 g body weight. All doses refer to the salt form of the drugs.

Behavioral Pattern Monitor Chambers

The Behavior Pattern Monitors (BPM) have been described in detail elsewhere [6]. Briefly, each chamber is a 30.5 by 61 by 38 cm black box with a stainless steel floor and wall touchplate (located 15 cm above the floor). Each chamber has three floor holes and seven wall holes. Holepokes are detected by an infrared photobeam in each hole. Rearings were detected when the animal made a connection, with his body, between the side of the wall and the floor of the box. A 4 by 8 perpendicular array of photobeams is used to localize the animal's position with 3.8 cm resolution. A microprocessor system checks the status of all beams every 100 msec. As changes occur in the photobeam patterns a data reading is taken with a time value recorded for each change.

Behavioral Measures

The dependent variables included the number of holepokes, rearings, and crossovers cumulated over 10 min intervals for 60 min. From the state of the 4 by 8 array of photobeams, the animal's (x,y) position was calculated and used to assign the rat to one of eight square "sectors," as described elsewhere [6]. "Crossovers" were defined as the total number of sector entries, and used as the most standard measure of horizontal locomotion and motor activity. As detailed elsewhere [6], the BPM system provides a wide variety of more detailed behavioral measures. Although these measures were examined, they are not included in this report because they were not necessary to adequately describe the nature of the drug effects.

Behavioral Testing

All behavioral testing was conducted during the dark phase of the animals' light/dark cycle. Animals were brought up to the laboratory one hour prior to behavioral testing. The first phase of this study established a dose-response curve for clenbuterol. Animals were injected 20 min before testing with either saline or clenbuterol (0.004, 0.01, 0.025, 0.05, 0.01 mg/kg). This dose range was selected because it had been shown to produce significant decreases in the locomotor activity of rats [8, 12, 14]. The dose-response assessments were conducted as two separate experiments, each including groups of control and 0.01 mg/kg clenbuterol animals. Since the controls and the effects of clenbuterol were similar in both experiments, the two experiments were combined. For each experiment, animals were randomly assigned to treatment groups of 10–12 rats each.

The second phase of this study examined the peripheral versus central nature of the decrease in motor activity produced by clenbuterol. An additional 60 rats were randomly assigned to 6 groups of 10 each for this experiment. Forty-five min before being placed in the BPM chambers, the animals received their first injection of either nadolol (10 mg/kg), or isoproterenol (0.4 mg/kg), 30 min after their first injection. An initial dose range of nadolol was chosen based on work comparing the effects of nadolol to propranolol on renal blood flow in rats [1]. The dose selected for use was then determined in pilot studies to be the highest dose which had no behaviorally suppressive effects by itself. The dose of isoproterenol was based on the report that isoproterenol was eight times less potent than clenbuterol on locomotor activity [3]. Nevertheless, some of the animals given isoproterenol in the absence of the nadolol pretreatment died within minutes of the injection, presumably from cardiovascular effects. The remaining animals appeared to be healthy and behaved normally at the time the test sessions began. Of the 10 rats assigned to the isoproterenol group, only 6 completed the experiment.

TABLE 1

EFFECTS OF CLENBUTEROL ON BEHAVIOR

Dose (mg/kg)	N	Crossovers	Holepokes	Rearings
0	19	2122 ± 75	252 ± 21	126 ± 13
0.004	9	1906 ± 125	192 ± 23*	127 ± 28
0.01	19	1695 ± 86*	190 ± 15*	90 ± 13
0.25	10	1361 ± 153*	162 ± 17*	72 ± 19*
0.05	9	609 ± 69*	52 ± 9*	28 ± 9*
0.1	9	410 ± 42*	25 ± 6*	13 ± 3*
0.5	9	445 ± 75*	26 ± 4*	11 ± 3*
1.0	10	253 ± 38*	21 ± 4*	6 ± 2*

*Signifies significant difference from vehicle control by Dunnett's t-test, $p<0.05$.

Statistics

Behavioral results were analysed by analysis of variance (ANOVA). The first analysis for each experiment used a two or three factor mixed-design ANOVA, the between subjects factor(s) being the comparisons between vehicle and nadolol pretreatments and/or between saline and either isoproterenol or clenbuterol, and the repeated measure being successive blocks of ten minutes. When significant interactions were found, additional ANOVAs were used to identify simple effects. Differences between specific dose groups and the corresponding controls were assessed with Dunnett's t-test.

RESULTS

Effects of Clenbuterol

Table 1 describes the effects of the beta-adrenergic agonist, clenbuterol, on the behavioral profile provided by the BPM. There were seven doses of clenbuterol examined. Locomotor activity (crossovers) was decreased significantly at a dose of 0.01 mg/kg and was consistently lowered further with each increase in dose, $F(7,86)=67.71$, $p<0.01$. Similarly, rearings, $F(7,86)=12.26$, $p<0.01$, and holepokes, $F(7,86)=31.30$, $p<0.01$, were significantly decreased at doses greater than 0.01 mg/kg, to the same degree and with the

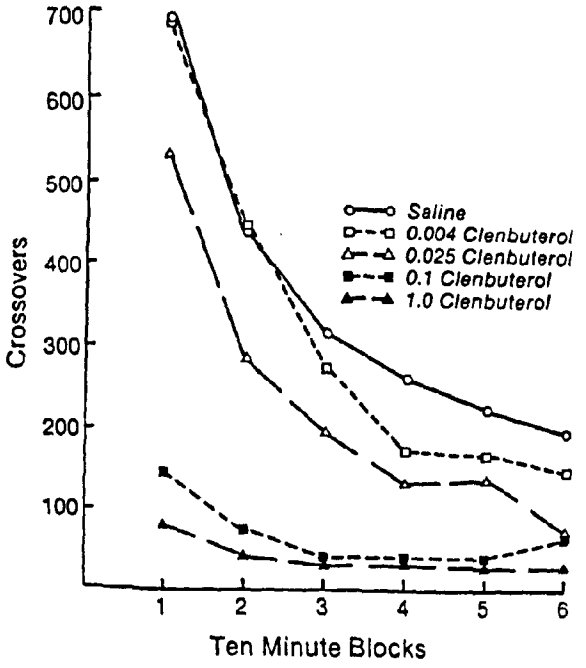

FIG. 1. Effects of various doses of clenbuterol on locomotor activity. Shown are the group means for crossovers across successive 10 min blocks of the hour test session for animals treated with vehicle or every other of the doses of clenbuterol tested.

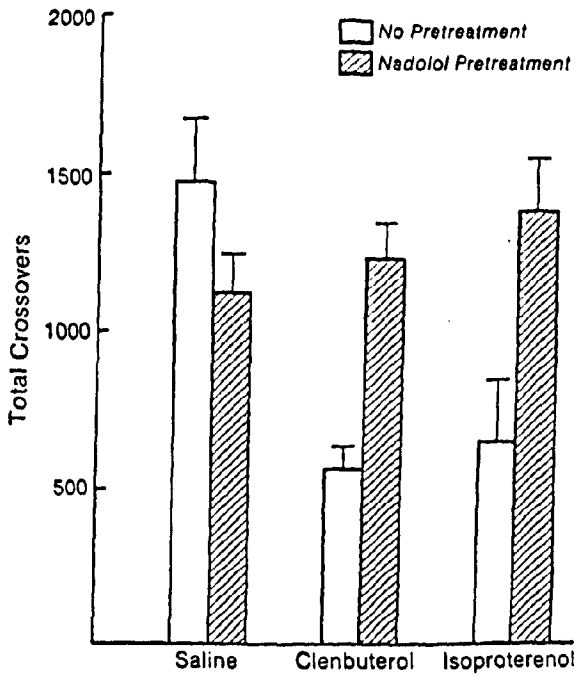

FIG. 2. Interaction of nadolol with isoproterenol or clenbuterol. Group means (±S.E.M.) for crossovers during the hour test sessions are shown for animals treated with saline, clenbuterol, or isoproterenol with or without the pretreatment with nadolol.

same time course as were crossovers. Figure 1 displays this decrease in crossovers over successive ten min intervals of the hour test session for every other dose tested.

Interaction of Nadolol With Beta Agonists

The overall ANOVA on crossovers for the second experiment revealed a significant interaction between the nadolol pretreatment and the beta-agonist treatment factors, $F(2,49)=7.04, p<0.001$, as illustrated in Fig. 2. A subsequent ANOVA assessing the effects of the nadolol pretreatment and the clenbuterol treatment on the total number of crossovers revealed a significant interaction between nadolol and clenbuterol, $F(1,36)=9.39, p<0.01$. The pretreatment with 10 mg/kg nadolol had no effect on crossovers by itself, $F(1,18)=2.28$, n.s., confirming a previous report [10]. As expected from the preceding experiment, the number of crossovers made by the animals treated with clenbuterol differed significantly from controls, $F(1,18)=17.55$, $p<0.01$. When pretreated with nadolol, however, the locomotor activity of the animals receiving clenbuterol did not differ significantly from saline controls, $F(1,18)=0.66$, n.s.

The two-factor ANOVA on the effects of nadolol and isoproterenol on total crossovers similarly revealed a significant interaction, $F(1,31)=9.66, p<0.01$. As with clenbuterol, isoproterenol produced a significant decrease in the number of crossovers, $F(1,14)=7.15$, $p<0.01$. The pattern of the isoproterenol-induced decrease in locomotor activity across successive 10 min intervals was virtually identical to that produced by clenbuterol (cf. Fig. 1). In contrast, animals pretreated with nadolol and then given isoproterenol did not differ significantly from controls, $F(1,17)=0.10$, n.s.

DISCUSSION

The results of this study indicate that the decrease in motor activity elicited by clenbuterol in rats is mediated by the activation of peripheral beta receptors. As expected from previous studies [2, 3, 12, 14], clenbuterol produced a dose-dependent decrease in locomotor activity throughout the hour test sessions. In addition, investigatory holepokes and rearings were also decreased by clenbuterol to the same extent and with the same time-course as the decrease in locomotor activity. However, a similar profile of behavioral effects was observed after injections of isoproterenol, a beta agonist which does not pass the blood-brain barrier. Though not conclusive, this similarity is consistent with the possibility that peripheral beta receptors contribute to the sedative effects of clenbuterol. In confirmation of a previous report [5], the effect of isoproterenol appears to be attributable to an activation of peripheral beta receptors, since it was blocked by the peripherally active beta antagonist, nadolol.

The stongest evidence against the hypothesis that the sedative effect of clenbuterol is mediated by central beta receptors is the demonstration that pretreatment with nadolol prevented any detectable effects of clenbuterol on locomotor activity. Despite the fact that nadolol does not enter the brain [10] and had no significant effects by itself at the dose used, it completely eliminated the decrease in motor activity normally produced by clenbuterol. As with isoproterenol, the amount of activity exhibited by the animals injected with both clenbuterol and nadolol was not different from that exhibited by control animals. Therefore, both the similarity in the effects of isoproterenol and clenbuterol and

the ability of a peripheral antagonist to block the effects of either agonist suggest that clenbuterol decreases motor activity by a peripheral mechanism.

It is important to note that the present results do not suggest that clenbuterol is devoid of effects on central beta receptors. Indeed, there is evidence that repeated administrations of clenbuterol produce changes in brain adrenergic receptors [9, 14, 15]. Further, since nadolol appears to be an antagonist at both beta-1 and beta-2 receptors, the present results are not inconsistent with the previous report that the selective beta-1 antagonist practolol did not prevent the sedation induced by clenbuterol [8]. The findings reported here do suggest that measures of locomotor activity or other behavioral measures which might be sensitive to sedative effects are not likely to be reflective of the putative central effects of beta receptor agonists such as clenbuterol.

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