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Pharmacological Characterization of the Discriminative Stimulus Effects of Clenbuterol in Rats

JAMES M. O'DONNELL

Department of Pharmacology, Louisiana State University School of Medicine, 1501 Kings Highway, Shreveport, LA 71130

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O'DONNELL, J. M. *Pharmacological characterization of the discriminative stimulus effects of clenbuterol in rats.* PHARMACOL BIOCHEM BEHAV **58**(3) 813–818, 1997.—The beta-2 selective adrenergic agonist clenbuterol produces discriminative stimulus effects in rats. Administration of beta adrenergic agonists that do not cross the blood–brain barrier well following peripheral administration either failed to substitute for clenbuterol or resulted in chance levels of drug-appropriate responding; this suggested central mediation of the effects of clenbuterol. This interpretation was supported by the finding that the centrally acting beta adrenergic antagonist propranolol antagonized the discriminative stimulus effects of clenbuterol more potently than did CGP-12177, a hydrophilic beta adrenergic antagonist that has been shown to have very limited central activity. Antagonism experiments using subtype-selective antagonists showed that the beta-2 selective antagonist ICI 118,551 more potently antagonized the discriminative effects of the training dose of clenbuterol than did the beta-1 selective antagonist betaxolol. The present results indicate that the discriminative stimulus effects of clenbuterol provide an in vivo index of activation of central beta-2 adrenergic receptors. © 1997 Elsevier Science Inc.

Beta adrenergic receptor Betaxolol CGP-12177 Clenbuterol Drug discrimination ICI 118,551 propranolol

THE beta-2 selective adrenergic agonist clenbuterol produces discriminative stimulus effects in rats. In an initial study, it was found that a dose of 0.1 mg/kg established discriminative control, that the discriminative stimulus was antagonized by the beta adrenergic antagonist propranolol, and that the effects of other beta-2 selective agonists generalized to the discriminative stimulus effects of clenbuterol (8). These data suggest that the discriminative stimulus effects of clenbuterol provide an in vivo index of activation of central beta-2 adrenergic receptors. Such an interpretation is supported by reports that clenbuterol is an agonist at beta-2 adrenergic receptors but has little, if any, efficacy at beta-1 adrenergic receptors (23). Further, it has been shown that, in contrast to classical catecholamine agonists such as epinephrine, norepinephrine, and isoproterenol, clenbuterol is centrally active following peripheral administration (2,15,19).

It also was found that administration of the beta-1 selective agonist prenalterol results in clenbuterol-appropriate responding in rats trained to discriminate clenbuterol from saline. Further, the antidepressants desipramine and phenelzine, which in neuropharmacological studies have been shown to affect the beta-1 subtype to a much greater extent than the beta-2 subtype (21,22), also substitute for clenbuterol (8). These data can be interpreted in two ways. First, it is possible that the discriminative stimulus effects of clenbuterol involve actions at beta-1 and well as beta-2 adrenergic receptors, possibly by an indirect mechanism (10–12). Second, the beta-1 selective agonists and antidepressants that have been shown to substitute for the discriminative stimulus effects of clenbuterol may do so by directly or indirectly stimulating beta-2 adrenergic receptors at the dose ranges tested. To begin to distinguish between these two possibilities, the discriminative stimulus effects of clenbuterol were characterized pharmacologically.

Experiments were carried out to assess whether the discriminative stimulus effects of clenbuterol were mediated by

central beta-2 adrenergic receptors. Assessment of central mediation was accomplished by examining the ability of catecholamine agonists that do not cross the blood–brain barrier well (2,19) to substitute for clenbuterol in a two-lever drug discrimination task. Further, the potencies of lipophilic (centrally and peripherally acting) and hydrophilic (peripherally acting primarily) antagonists to antagonize the discriminative stimulus effects of clenbuterol were compared (12,26). Assessment of which subtype of the beta adrenergic receptor mediates the discriminative stimulus effects of clenbuterol was accomplished by comparing the antagonistic potencies of the beta-1 selective antagonist betaxolol and the beta-2 selective antagonist ICI 118,551 (16,19).

METHOD

Subjects

Male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 350–450 g were housed singly in polycarbonate cages and kept in a room maintained at a constant temperature and humidity and illuminated 12 h per day. Rat had free access to food, except during behavioral training and test sessions, but were maintained under a regimen of restricted access to water. Water was provided after each daily session to maintain body weights.

Discrimination Training and Testing

Twenty rats that had not received any other drug treatment previously were trained to respond for water reinforcement using Coulbourn Model E10-10 operant chambers that contained two levers, a water access port capable of delivering 0.04 ml of water as a reinforcer, a houselight, and a fan that provided ventilation and a masking noise. Schedule contingencies were programmed on a Micro PDP 11/73 computer using the SKED-11 operating system (25). After acquisition of lever-pressing behavior, rats were gradually shifted to a fixed-ratio 10 schedule; on a daily basis, reinforcement was alternately made available for completion of the schedule requirement on either the right or left levers. Following the development of stable responding under this schedule, rats were trained in daily 10-min sessions to discriminate 0.1 mg/kg clenbuterol from the saline vehicle as described previously (8). Discrimination training continued until rats reached the performance criterion of completion of the fixed-ratio 10 requirement on the injection-appropriate lever (i.e., correct lever selection) on 9 of 10 consecutive training sessions.

Once clenbuterol discrimination was well established, a series of generalization and antagonism tests were conducted during weekly test sessions using groups of at least five rats that met the performance criterion at the time of the test; training sessions continued on the remaining days each week. On test days, rats were administered either a beta adrenergic agonist or antagonist/agonist combination and tested for lever selection. The selected lever was defined as that lever on which the fixed-ratio 10 requirement was first met. After lever selection, reinforcement was not provided, regardless of which lever was selected, and each rat was removed from its experimental chamber. If the fixed-ratio 10 requirement was not completed for either lever within 10 min, the session was terminated.

Data Analysis

Drug discrimination results are expressed as the percentage of rats selecting the clenbuterol-appropriate lever; rats not completing the fixed-ratio requirement on either lever within the 10 min were excluded from the analysis. To calculate ED_{50} values for the antagonists, the dose–response functions were subjected to nonlinear regression analysis (3), as described previously (13,14).

Drugs

Clenbuterol, epinephrine, isoproterenol, norepinephrine, and propranolol were purchased from Sigma Chemicals (St. Louis, MO). Betaxolol (LERS Synthelabo, Paris, France), CGP-12177 (Ciba-Geigy Pharmaceuticals, Basel, Switzerland), and ICI 118,551 (Zeneca Pharmaceuticals, formerly ICI Pharmaceuticals, Cheshire, UK) were provided as gifts by their manufacturers. All drugs were hydrochloride salts, except isoproterenol, which was a bitartrate salt, and were dissolved in a 0.9% sodium chloride solution, which served as the drug vehicle; doses shown are those of the free bases. Clenbuterol and the other beta adrenergic agonists (or the saline vehicle) were administered 30 min before training and test sessions, except as otherwise indicated in the Results section. The antagonists betaxolol, ICI 118,551, propranolol, and CGP-12177 were administered 35 min before test sessions. Drug and vehicle solutions were administered IP at a volume of 1 ml/kg body weight.

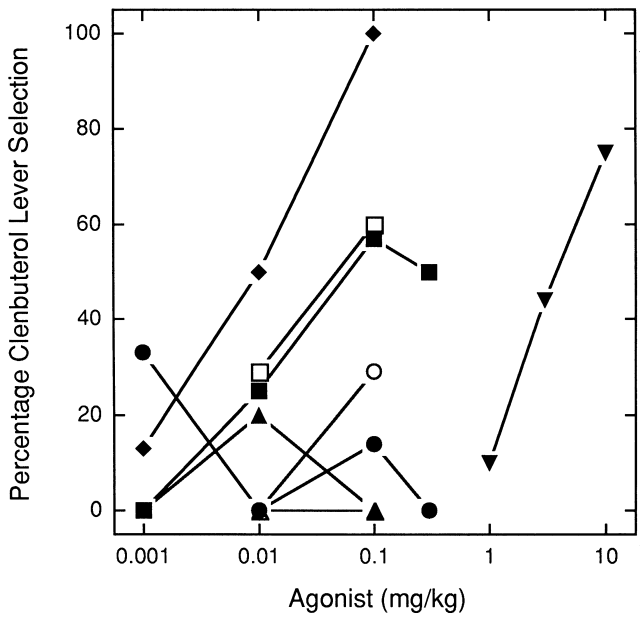

FIG. 1. Discriminative stimulus generalization tests with beta adrenergic agonists. Lever selection is shown as a function of dose of clenbuterol (◆), dobutamine (▼), norepinephrine (●, ○), epinephrine (▲, △), and isoproterenol (■, □). Filled symbols show data when agonists were administered 30 min prior to the test session. Open symbols show data when agonists were administered 10 min prior to the test session. Data are percentage of 6–10 rats selecting the clenbuterol-appropriate lever as defined by completion of the fixed-ratio 10 requirement. At the highest doses tested, the number of rats that failed to make a lever selection for each drug were: clenbuterol, 0 of 8 rats tested; dobutamine, 3 of 11; isoproterenol, 3 of 6; epinephrine, 4 of 6; norepinephrine, 0 of 7. At the second to lowest doses tested, administration of the drugs did not result in the failure of rats to make lever selections.

RESULTS

After establishment of discrimination, administration of clenbuterol resulted in dose-dependent selection of the clenbuterol-appropriate lever (Fig. 1). At a dose of 0.01 mg/kg, clenbuterol administration produced 50% drug lever selection; administration of the training dose of 0.1 mg/kg resulted in 100% selection of the clenbuterol-appropriate lever. Administration of the beta-1 selective adrenergic agonist dobutamine also resulted in dose-dependent selection of the drug lever (Fig. 1). However, even at a dose of 10 mg/kg, only 75% of the rats selected the clenbuterol-appropriate lever; administration of a threefold higher dose resulted in the failure of rats to complete the fixed-ratio requirement on either lever within the ten minute test session.

Of the other agonists tested, epinephrine, norepinephrine, and isoproterenol, none produced more than 60% clenbuterol lever selection (Fig. 1). The greatest generalization was for the effects of isoproterenol, which at doses of 0.1 and 0.3 mg/kg resulted in 50–60% selection of the clenbuterol-appropriate lever. The effects of the agonists were similar whether they were administered either 10 or 30 min prior to the test session. When any of these agonists were administered at doses threefold higher than those shown in Fig. 1, nearly all rats failed to complete the fixed-ratio requirement on either lever.

Administration of either the lipophilic beta adrenergic antagonist propranolol or the hydrophilic beta adrenergic antagonist CGP-12177 5 min prior to clenbuterol administration antagonized the discriminative stimulus effects of clenbuterol in a dose-dependent manner (Fig. 2). Administration of 1 mg/kg propranolol prior to 0.1 mg/kg clenbuterol resulted in 0% selection of the clenbuterol-appropriate lever. By contrast, administration of 10 mg/kg CGP-12177 prior to clenbuterol still resulted in approximately 30% selection of the clenbuterol-appropriate lever. ED_{50} values for antagonism of the discriminative stimulus effects of clenbuterol by propranolol and CGP-12177 are 0.005 mg/kg and 1.34 mg/kg, respectively (Table 1).

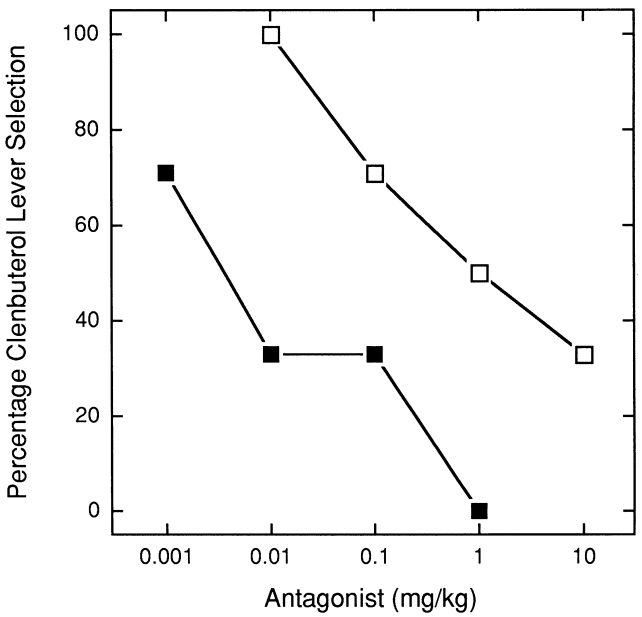

FIG. 2. Discriminative stimulus antagonism tests with beta adrenergic antagonists that differ in lipophilicity and apparent central activity. Lever selection is shown as a function of dose of propranolol (■) or CGP-12177 (□). Data are percentage of five to seven rats selecting the clenbuterol-appropriate lever as defined by completion of the fixed-ratio 10 requirement.

TABLE 1

ED_{50} VALUES FOR ANTAGONISM OF THE DISCIMINATIVE STIMULUS EFFECTS OF CLENBUTEROL AND FOR INHIBITION OF ^{125}I-PINDOLOL BINDING IN VIVO BY PROPRANOLOL AND CGP-12177

Measure	ED_{50} (mg/kg)		Ratio‡
	CGP-12177	Propranolol	
Antagonism of clenbuterol discrimination*	1.34 ± .29	0.005 ± .002	268
Inhibition of ^{125}I-pindolol binding in cerebellum in vivo†	2.43	0.02	122
Inhibition of 125I-pindolol binding in lung in vivo†	0.04	0.03	1.3

*Values shown are doses of the antagonists that antagonize the discriminative stimulus effects of 0.1 mg/kg clenbuterol by 50%, as calculated by nonlinear regression analysis.

†Values shown are doses of the antagonists that inhibit ^{125}I-pindolol binding in vivo by 50% in lung (peripheral receptors) and cerebellum (central receptors). ED_{50} values are taken from (15).

‡Values shown are the ratios of the ED_{50} values for CGP-12177 and propranolol for each experimental measure. These provide indices of the relative potency of the antagonists.

Administration of either the beta-1 selective adrenergic antagonist betaxolol or the beta-2 selective adrenergic antagonist ICI 118,551 antagonized the discriminative stimulus effects of clenbuterol in a dose-dependent manner (Fig. 3). Near complete antagonism of the discriminative stimulus effects of clenbuterol was observed after pretreatment with 0.1–1 mg/kg ICI 118,551. By contrast, within this dose range, clenbuterol administration still resulted in 40–88% selection of the clenbuterol-appropriate lever following pretreatment with betaxolol. Calculation of ED_{50} values for antagonism of the discriminative stimulus effects of clenbuterol by ICI 118,551 and betaxolol showed the beta-2 selective antagonist to be about 70-fold more potent than the beta-1 selective antagonist (Table 2).

DISCUSSION

Overall, the present results suggest that the discriminative stimulus effects of clenbuterol are mediated predominantly by central beta-2 adrenergic receptors. Evidence for central mediation is provided by the results of generalization tests with beta adrenergic agonists and antagonism tests with propranolol and CGP-12177. In rats trained to discriminate 0.1 mg/kg clenbuterol from saline, three beta adrenergic agonists (epinephrine, norepinephrine, isoproterenol), which have been shown not to cross the blood–brain barrier well following systemic administration (2,19), produced either saline-appropriate lever selection or lever selection near the chance level of 50%. Administration of higher doses of these agonists disrupted performance to the point that very few rats actually completed the fixed-ratio requirement.

The results of antagonism studies with propranolol and CGP-12177 also supported the conclusion that the discriminative stimulus effects of clenbuterol are mediated by central

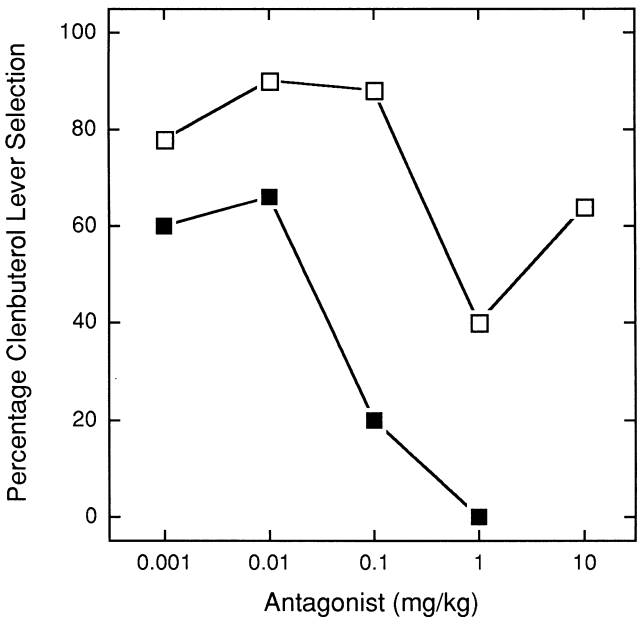

FIG. 3. Discriminative stimulus antagonism tests with subtype-selective beta adrenergic antagonists. Lever selection is shown as a function of dose of the beta-2 selective antagonist ICI 118,551 (■) or the beta-1 selective antagonist betaxolol (□). Data are percentage of 5–14 rats selecting the clenbuterol-appropriate lever as defined by completion of the fixed-ratio requirement.

beta adrenergic receptors. These two antagonists exhibit similar affinity for beta adrenergic receptors in vitro (K_I values of about 1 nM) (15). In addition, following systemic administration, propranolol and CGP-12177 inhibit ^{125}I-pindolol binding to heart and lung in vivo with similar potency (ED_{50} values of about 0.03 mg/kg). However, the hydrophilic antagonist CGP-12177 crosses membranes poorly (26); thus, after systemic administration, it crosses the blood–brain barrier less readily than does propranolol. This is evidenced by CGP-12177 being approximately 100-fold less potent than propranolol for inhibition of ^{125}I-pindolol binding in cerebral cortex and cerebellum in vivo (15). It was found that propranolol was greater than 200 times more potent than CGP-12177 for antagonizing the discriminative stimulus effects of clenbuterol (Table 1). This potency difference is consistent with the potency difference for the interaction of these antagonists with central, rather than peripheral, beta adrenergic receptors, suggesting that the discriminative stimulus effects of clenbuterol result from an interaction of the drug with beta adrenergic receptors in the brain. Because both CGP-12177 and propranolol exhibit similar affinity for beta-1 and beta-2 adrenergic receptors, their antagonism provides no indication of the receptor subtype involved in the actions of clenbuterol. Further, the interaction of CGP-12177 with the beta-3 subtype of the receptor likely contributes little to the present results, because this subtype appears not to be expressed in brain (5,6).

The results of antagonism experiments with betaxolol and ICI 118,551 suggest that beta-2 adrenergic receptors predominantly mediate the discriminative stimulus effects of clenbuterol. ICI 118,551 is approximately 400-fold more potent than betaxolol at inhibiting ^{125}I-pindolol binding to beta adrenergic receptors in cerebellum (mostly beta-2 subtype) (24) in vivo. By contrast, betaxolol is about 12-fold more potent than ICI 118,551 at inhibiting ^{125}I-pindolol binding to beta adrenergic receptors in cerebral cortex (mostly beta-1 subtype) (24) in vivo (2). The finding that ICI 118,551 is about 70-fold more potent than betaxolol for antagonism of the discriminative stimulus effect of clenbuterol (Table 2) suggests that an interaction with the beta-2 subtype is predominantly responsible for mediation of this effect. The ability of a number of centrally active, beta-2 selective agonists to potently and fully substitute in rats trained to discriminate clenbuterol supports this conclusion (8). The present data also are consistent with neuropharmacological data on clenbuterol (16,23,27). It also has been found that repeated treatment with clenbuterol, which downregulates beta adrenergic receptors and abolishes the behavioral effects of beta-2 selective agonists (16), greatly reduces the discriminative stimulus effect of 0.03 mg/kg clenbuterol (Makhay and O'Donnell, unpublished data).

The finding that the discriminative stimulus effects of clenbuterol are mediated by central beta-2 adrenergic receptors is in agreement with the results of studies examining other be-

TABLE 2

ED_{50} VALUES FOR ANTAGONISM OF THE DISCIMINATIVE STIMULUS EFFECTS OF CLENBUTEROL AND FOR INHIBITION OF ^{125}I-PINDOLOL BINDING IN VIVO BY SUBTYPE-SELECTIVE ANTAGONISTS

Measure	ED_{50} (mg/kg)		Ratio‡
	Betaxolol	ICI 118,551	
Antagonism of clenbuterol discrimination*	0.67 ± .16§	0.009 ± .004	74
Inhibition of ^{125}I-pindolol binding in cerebellum in vivo†	8.6	0.02	430
Inhibition of ^{125}I-pindolol binding in lung in vivo†	0.7	8.7	0.08

*Values shown are doses of the subtype-selective antagonists that antagonize the discriminative stimulus effects of 0.1 mg/kg clenbuterol by 50%, as calculated by nonlinear regression analysis.

†Values shown are doses of the subtype-selective antagonists that inhibit ^{125}I-pindolol binding in vivo by 50% in cerebellum (mostly beta-2 subtype) and cerebral cortex (mostly beta-1 subtype). ED_{50} values are taken from (2).

‡Values shown are the ratios of the ED_{50} values for betaxolol and ICI 118,551 for each experimental measure. These provide indices of the relative potency of the antagonists.

§The ED_{50} value for betaxolol was determined with the 10 mg/kg data point excluded, because inclusion of this point prevented reliable estimation.

havioral effects of clenbuterol. The results of studies comparing antagonistic potencies of betaxolol and ICI 118,551 and propranolol and CGP-12177 indicate that the antidepressant-like effects of clenbuterol on behavior maintained under a differential-reinforcement-of-low-rate schedule are mediated by central beta adrenergic receptors (15,16). Similarly, the effects of clenbuterol on behavior maintained under a multiple fixed-interval, fixed-ratio schedule appear to be centrally mediated (14). Similar evidence for central mediation of the effects of the beta-2 selective adrenergic agonist albuterol on schedule-controlled behavior and food intake also has been reported (1,17). While these behavioral and discriminative stimulus effects of clenbuterol and related drugs appear centrally mediated, beta adrenergic agonists can produce behavioral changes as a result of interactions with peripheral receptors. The effects of clenbuterol on locomotor activity are equally well antagonized by centrally and peripherally acting beta adrenergic antagonists (4,18). Consistent with this finding, isoproterenol, which does not appear to be centrally active following peripheral administration (2,19), potently and efficaciously reduces locomotor activity (18).

The present results indicate that the discriminative stimulus effects of clenbuterol provide an in vivo index of activation of central beta-2 adrenergic receptors. Thus, it is likely that generalization of the effects of other drugs to the discriminative stimulus effects of clenbuterol would suggest that these drugs stimulate, by some mechanism, central beta-2 adrenergic receptors. Substitution by the beta-1 selective agonists dobutamine and prenalterol (8), are consistent with the finding that their beta-1 selectivity is only relative and that, as doses are increased, stimulation of the beta-2 subtype likely results. Generalization of the effects of desipramine and phenelzine to the discriminative stimulus effects of clenbuterol is somewhat more surprising because norepinephrine, presumably the endogenous mediator of the effects of these antidepressants, is 100-fold less potent at beta-2 than beta-1 subtypes of the receptor (9,20). However, data suggesting an involvement of beta-2 adrenergic receptors in the mediation of a behavioral effect of desipramine have been reported (7). It remains unclear whether stimulation of central beta-2 adrenergic receptors, by some mechanism, is an effect shared by other antidepressant drugs.

ACKNOWLEDGEMENTS

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