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# Effects of Various Catecholamine Receptor Antagonists, Muscle Relaxation and Physical Hindrance on Shuttlebox Self-Stimulation

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LIEBMAN, J. M., N. HALL AND J. PROWSE. *Effects of various catecholamine receptor antagonists, muscle relaxation and physical hindrance on shuttlebox self-stimulation.* PHARMAC. BIOCHEM. BEHAV. 16(5) 785–790, 1982.—Certain drugs, particularly clozapine and clonidine, have been reported to increase selectively the latency to initiate brain stimulation (the ON latency) in a shuttlebox test of self-stimulation, suggesting a preferential attenuation of the "reward" component. The pharmacological selectivity of this reported effect was systematically evaluated. At doses that blocked bar-pressing self-stimulation, metoclopramide (3 mg/kg), prazosin (3 mg/kg), clonidine (0.1 mg/kg), clozapine (3 mg/kg) and haloperidol (0.3 mg/kg), all elevated the ON latency to a greater extent than the OFF latency. Methocarbamol (200 mg/kg), a muscle relaxant, also elevated the ON latency preferentially but the magnitude of this preferential effect was smaller than that produced by the other drugs. A hurdle in the center of the shuttlebox increased the ON and OFF latencies nonselectively. The shuttlebox procedure does not clearly discriminate among various substances that interfere with noradrenergic or dopaminergic neurotransmission, but the common profile produced by these substances is distinguishable to some degree from simple motor disruption.

Self-stimulation Shuttlebox Haloperidol Clozapine Clonidine Metoclopramide Prazosin Methocarbamol

DRUGS may alter bar-pressing rates for intracranial self-stimulation by changing the effectiveness of rewarding brain stimulation or, alternatively, by directly compromising motor capability [8, 9, 10, 21]. In response to these generally acknowledged limitations upon the interpretation of bar-pressing self-stimulation data, alternative procedures have been developed for the assessment of drug effects [1, 6, 18, 19]. Among these, the shuttlebox self-stimulation test of Atrens [1] appears to be a relatively simple and rapid procedure. In this test, the animal is allowed to control the onset and duration of brain stimulation separately by interrupting two photocell beams located at opposite ends of a shuttlebox. The latency to initiate brain stimulation is termed the ON latency and is hypothesized to vary inversely with reward value of stimulation [1]. The OFF latency is that required to terminate rewarding brain stimulation. If the OFF latency fails to change concurrently with the ON latency, then it is difficult to attribute changes in the ON latency to performance variables.

Using this test procedure, drug studies have been performed that address the controversial issue of whether self-stimulation is directly affected by drugs that alter noradrenergic and/or dopaminergic neurotransmission [1, 7, 12, 13]. It has been claimed that clonidine and clozapine selectively increase the ON latencies, whereas haloperidol non-selectively elevates both ON and OFF latencies [1,12]. The reported experimental discrimination between haloperidol and clozapine is of particular interest in view of the differential ability of these two antipsychotics to elicit extrapyramidal symptoms [4]. A subsequent report from another laboratory, however, found that haloperidol did have a selective effect upon ON latencies [7]. To reconcile these discrepancies, we have systematically compared the effects of haloperidol, clozapine and clonidine with those of a selective dopamine receptor antagonist, metoclopramide [12] and a selective alpha-1 adrenoreceptor antagonist, prazosin [5]. The effects of these drugs on bar-pressing self-stimulation were also examined to determine the dose relationships between impairment of these two measures of self-stimulation.

The present investigations also address the criticism [9] that the ON latency may simply be intrinsically more sensitive to drug-induced performance impairment than the OFF latency. If this were so, then simple physical hindrance of responding would elevate the ON latency selectively, as would a drug that had primarily muscle relaxant effects. We have, therefore, examined the effects on responding of interposing a hurdle in the center of the shuttlebox, as well as those of treatment with methocarbamol, a muscle relaxant in clinical use [3].

## METHOD

### *Animals and Surgical Procedures*

Male Fischer (F-344, Charles River) rats (250–300 g) were anesthetized (IM) with 20 mg ketamine HCl to which acepromazine (0.75 mg/ml) had been added to induce muscle relaxation. Stainless steel bipolar electrodes which were pre-attached to plastic connectors (Plastic Products, Roanoke, VA) were stereotaxically implanted in the lateral hypothalamus with the tooth bar at 2.4 mm ventral to horizontal zero to conform with the stereotaxic atlas of König and Klippel [14]. Acrylic cement secured the implanted electrode-plug assembly to stainless steel screws that had been threaded into the skull. Animals were allowed to recover for at least one week before the initiation of behavioral testing.

### *Behavioral Procedures*

The shuttlebox procedures were similar to those of Atrens [1] and have been previously described [15]. Animals were tested in a large chamber (47×28×28 cm) containing a grid floor. At each end of the chamber, a photocell beam was positioned 8 cm from the end wall and 4.5 cm above the grid floor. An Inter-Act (BRS/LVE, Beltsville, MD) computer system controlled the experiment such that interruption of one photocell beam (the ON beam) caused biphasic rectangular pulses to be delivered continuously through the implanted electrode until the other photocell beam (the OFF beam) was interrupted by the rat. Brain stimulation was delivered by a Haer 4 bp stimulator according to the following parameters: pulse duration 0.4 msec, pulse frequency 100 Hz, current intensity 40 to 200 $\mu$A.

After initial training, rats underwent daily, 10-min test sessions. The first beam which the rat interrupted after entry into the shuttlebox automatically became the ON beam. Three "warm-up" crossing cycles, in which the rat sequentially interrupted both beams during each cycle, were allowed before data collection began. The total number of crossing cycles in the next 10 min was recorded, as were the cumulative latencies to break the ON and the OFF beams. The task was programmed so that if the rat allowed either latency to reach 60 sec at any time, the ON and OFF beams were automatically reversed. Whenever this happened, current was immediately delivered or switched off, depending on the type of latency which the rat had allowed to reach the 60 sec criterion.

After initial training, a total of 29 rats were used for drug experiments. In these rats, current intensity was individually adjusted so as to yield between 35 and 80 crossing cycles per session. Rats received drug treatments only after at least two days of stable baseline responding occurred within these limits. An additional constraint was that drug data were not collected if either the mean ON or OFF latency was less than 2 sec. The reason for this additional criterion was that asymptotic latencies (i.e., 1 sec or less) were found in pilot studies to be refractory to drug-induced effects. This phenomenon is also evident in data presented by Edwards *et al.* [7].

In one experiment, a solid hurdle constructed of acrylic plastic (5 or 10 cm high) was placed across the center of the shuttlebox in lieu of drug treatment. The rat had to climb over this hurdle to get from one end of the shuttlebox to the other. Separate groups of rats underwent testing with the 5 and 10 cm hurdles, respectively. No rat was tested more than once with the hurdle in the shuttlebox.

A separate group of rats (n=42) was used for bar-pressing self-stimulation experiments. After initial training to bar-press on a continuous reinforcement schedule for brain stimulation (100 msec stimulus trains, biphasic square wave pulses, pulse width 0.05 msec), these rats were tested in two daily 15-min test sessions that were separated by 3 hr (except as noted below). Current intensity was progressively reduced in succeeding sessions until a submaximal response rate was consistently elicited, typically 300 to 800 bar-presses per session. Drug treatments were administered after at least three consecutive days of stable baseline responding occurred within these limits.

### *Drug Treatments*

All drugs were administered intraperitoneally in a volume of 1 cc/kg, except that a volume of 2 cc/kg of methocarbamol was necessary to avoid excessive viscosity. Methocarbamol and haloperidol were administered 1 hr prior to shuttlebox testing. All other drugs were given 30 min before the shuttlebox session or the first bar-press session on a given day. Each rat received all doses of a given drug in the shuttlebox experiments. In the bar-pressing experiments, either a within-subject or an incomplete block design was employed. At least 5 days elapsed between successive drug treatments in each rat.

To assess the possible contribution of a "first-dose" hypotensive phenomenon [5] to the behavioral effects of prazosin, tolerance to prazosin's effects on bar-pressing self-stimulation was assessed in a separate experiment. In this experiment, an additional group of animals received prazosin (3 mg/kg) once daily for four consecutive days, and was tested 30 min after each treatment. Baseline responding during the day preceding the first of these treatments served as a pre-drug control.

Clonidine and metoclopramide were dissolved in normal saline for injection, and haloperidol, methocarbamol and prazosin were prepared in a colloidal cornstarch suspension containing 5% PEG-400 and 0.34% Tween 80. Clozapine was dissolved in 0.1 N tartaric acid and saline was added to bring the solution to the desired concentration. Drug sources were: clonidine hydrochloride (Boehringer-Ingelheim, Ridgefield, CT), clozapine (Sandoz, East Hanover, NJ), haloperidol (McNeil, Fort Washinton, PA), methocarbamol and metoclopramide (A. H. Robins, Richmond, VA), prazosin (Pfizer, Groton, CT). Where applicable, all doses were expressed as the respective salts.

### *Analysis of Data*

The cumulative ON and OFF latencies for each drug during a given experimental session were divided by the total number of response cycles to yield mean ON and OFF latencies, respectively. Drug effects on shuttlebox performance were assessed by comparing mean ON and OFF latencies with those during the preceding baseline session. To determine whether significant dose-response relationships existed for the drug-induced changes in ON and OFF latencies, regression analyses were performed separately on the ON and OFF latency data. Whenever these analyses indicated a significant dose-response relationship, the trend test [2] was then applied to identify doses that produced a significant ($\alpha$=0.05) increase in latency over the pre-drug baseline.

It was also of interest to determine whether the magnitude of the drug-induced increase in the ON latency significantly exceeded that in the OFF latency. For this purpose, whenever

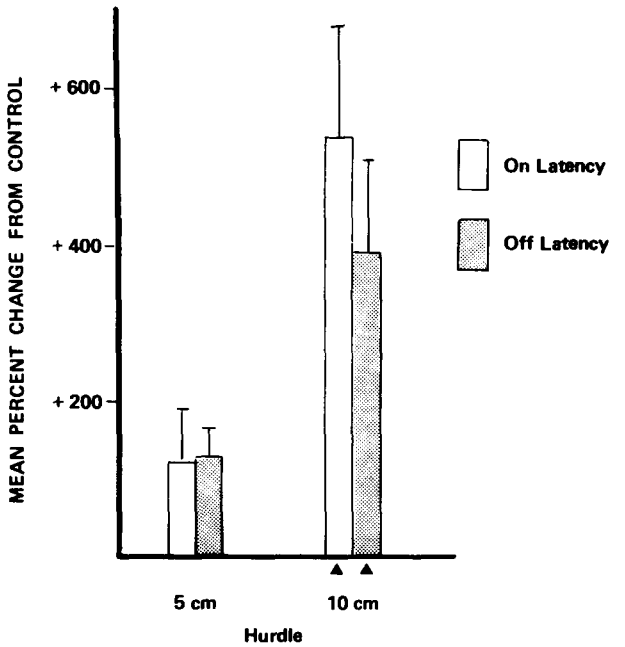


FIG. 1. Effects on shuttlebox self-stimulation performance of a hurdle in the center of the shuttlebox. Bars indicate mean (±S.E.) percent increase in latency from pre-drug baseline. ▲ Significantly different from pre-drug baseline by the trend test, $p<0.05$. N=9.

analysis of variance indicated a significant main effect of type of latency and of drug dose, matched pair $t$-tests were performed that compared the percent increases in ON and OFF latencies at given drug doses.

Regression analyses were performed on drug-induced percent changes in bar-pressing rates from the pre-drug baseline session. The effects of repeated administration of prazosin were evaluated by analysis of variance, followed by Tukey's (h.s.d.) multiple comparison procedure [20].

*Histology*

To verify that the electrode placements were centered in the posterior lateral hypothalamus, representative rats were sacrificed at the conclusion of experimentation for histological evaluation. Following transcardial perfusion with saline and Formalin, the brains were removed, allowed to stand in Formalin for at least 24 hr, then sectioned and stained using the cresyl violet or the Weil method.

## RESULTS

*Shuttlebox Self-Stimulation*

The mean baseline latencies for each treatment group prior to drug treatment ranged from 4.2 to 6.3 sec (ON latency) and from 5.1 to 7.5 sec (OFF latency). In no case did the group baseline ON and OFF latencies differ significantly prior to a given drug treatment ($p>0.30$ for all comparisons, matched pair $t$-test, two-tail).

Both the ON and OFF latencies were significantly elevated by the presence of a 10 cm barrier (hurdle) in the center of the shuttlebox, as compared with baseline values during the session that preceded this manipulation (Fig. 1). Observations indicated that these elevations in latencies largely reflected longer delays by the rats prior to initiating crossings of the hurdle. Once a crossing was initiated, the rat typically required only two to four sec to complete it. The percent increase in the ON latency did not differ significantly from that in the OFF latency. The effects of the 5 cm barrier were of smaller magnitude and failed to reach statistical significance.

Moderate doses of haloperidol (0.3 mg/kg), clozapine (3.0 mg/kg), metoclopramide (3.0 mg/kg), prazosin (3.0 mg/kg) and clonidine (0.1 mg/kg) caused the ON latency to increase significantly from baseline without altering to a statistically significant degree the OFF latency (Figs. 2 and 3). At these doses (except for haloperidol at 0.3 mg/kg), the drug-induced increases in the ON latency also exceeded the smaller (nonsignificant) increases in OFF latencies. Slight increases in ON latencies were also noted after lower doses of haloperidol (0.1 and 0.03 mg/kg).

When drug doses three times greater than these effective doses were administered, clozapine, haloperidol, metoclopramide and prazosin all elevated both the ON and OFF latencies to values that exceeded four times baseline (data not shown). These doses appeared to cause nonspecific performance deficits, as indicated by the additional observation that at least two animals in each group virtually ceased to respond after treatment. Clonidine's effects on the OFF latency at the higher dose of 0.3 mg/kg were relatively moderate by comparison with its marked elevation of ON latencies, and the magnitude of the increase in ON latencies again exceeded that in the OFF latencies (Fig. 3). However, at this dose of clonidine, the increase in the OFF latency did reach statistical significance when compared with baseline.

An intermediate dose of methocarbamol (200 mg/kg) caused the ON latency, but not the OFF latency, to exceed pre-drug baselines significantly (Fig. 4). Although the percent increase in ON latency at this dose was relatively small, it also significantly exceeded the corresponding percent increase in the OFF latency. At 300 mg/kg, several animals virtually ceased responding and both latencies were elevated to more than five times baseline, indicating a nonspecific effect (data not shown).

Bar-pressing self-stimulation was reduced in a dose-related fashion by all of the drugs tested (Table 1). The lowest dose that was found to reduce bar-pressing by at least 80% from baseline generally corresponded to the dose that increased ON latencies selectively. The one exception was clozapine, which produced a 52% reduction at 3 mg/kg. Again, the maximum effect of methocarbamol was seen within a narrow range of doses.

The suppressant effects of prazosin (3 mg/kg) on bar-pressing self-stimulation remained statistically significant over four days of repeated treatment, by comparison with baseline. Response rate on Day 1 was reduced 88% from baseline, that on Day 2 by 59% and that on Day 4 by 73%. This slight tendency towards tolerance failed to reach statistical significance at the 0.05 level.

*Histology*

Electrode placements were evaluated in 13 of the 29 rats that served in the shuttlebox experiments, and in 17 of the 42 rats in the bar-pressing experiments. These rats were representative of the various experimental treatment groups. Placements were located between the +2970 and +4620 frontal planes in the König and Klippel atlas [14], and ranged from 0.8 to 1.8 mm lateral to midline. With the exception of

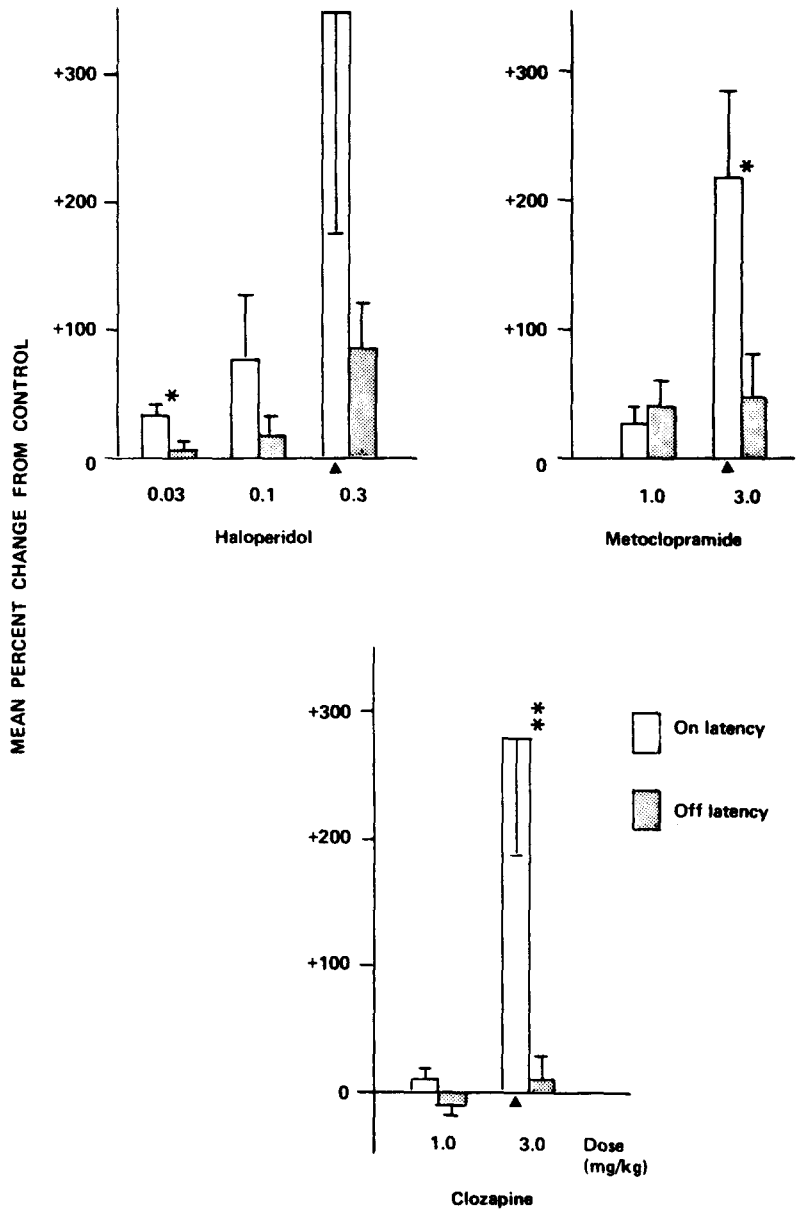


FIG. 2. Effects of haloperidol, metoclopramide and clozapine on shuttlebox self-stimulation. *Percent increase in ON latency was significantly greater than that in OFF latency, $p<0.05$; **$p<0.01$; ***$p<0.001$ (matched pair $t$-test, two-tailed). See legend, Fig. 1, for further explanation of format. N=8 per group.

five placements located slightly dorsomedial to the zona incerta, these placements were well within the lateral hypothalamus.

## DISCUSSION

Blockade of either alpha-1 adrenoreceptors (by prazosin) or dopaminergic receptors (by haloperidol or metoclopramide) produced very similar effects in the shuttlebox self-stimulation test. Characteristically, the ON latency was preferentially elevated by a drug dose that had been just sufficient to reduce bar-pressing self-stimulation by 80% or more. Such a dose increased the ON latency by three- to four-fold without causing statistically significant increases in the OFF latency over pre-drug baseline levels. The pre-drug ON and OFF baseline latency values were equivalent in all of these experiments. The importance of equating baseline performance when comparing operants has been emphasized previously [9].

The effects of haloperidol are in agreement with those reported by Edwards *et al.* [7], and fail to confirm a previous report [1] that haloperidol was less selective than clozapine in this test. The similarity of metoclopramide's effects to those of clozapine also indicates that the shuttlebox self-stimulation test does not clearly discriminate clozapine from strong dopamine receptor antagonists.

Prazosin, a selective alpha-1 adrenoreceptor antagonist,

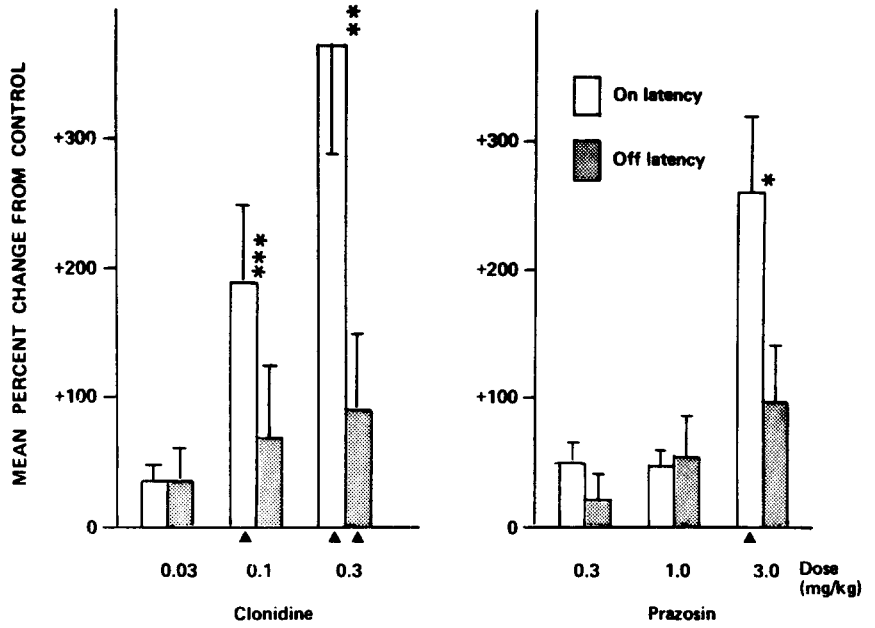


FIG. 3. Effects of clonidine and prazosin on shuttlebox self-stimulation performance. See legends, Figs. 1 and 2 for explanation of format. N=7 for clonidine; N=8 for prazosin.

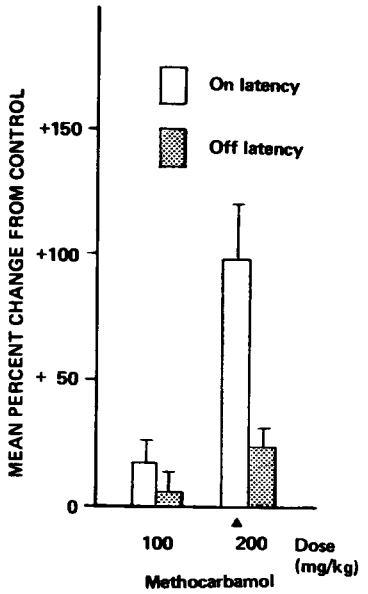


FIG. 4. Effects of methocarbamol on shuttlebox self-stimulation performance. See legends, Figs. 1 and 2 for explanation of format. N=8.

blocks brain alpha-1 receptors when administered systemically [5]. In the shuttlebox experiment, this drug yielded a profile very similar to that produced by haloperidol. A previous report [7] has indicated that another alpha-adrenoreceptor blocker, phentolamine, also produces selective increases in shuttlebox ON latencies. Relatively little tolerance to the effects of prazosin on bar-pressing self-stimulation was evident over a four-day treatment period. Thus, the effects of prazosin on bar-pressing and shuttlebox self-stimulation are

TABLE 1

EFFECTS OF METHOCARBAMOL, HALOPERIDOL, METOCLOPRAMIDE, CLOZAPINE, CLONIDINE AND PRAZOSIN ON BAR-PRESSING SELF-STIMULATION

| Drug | Dose mg/kg IP | Percent Change in Bar-Pressing per 15 Min Session* |
|---|---|---|
| Methocarbamol | 50 | − 23 ± 13 |
| (n=6) | 100 | − 51 ± 13 |
| | 200 | −100 ± 0 |
| Haloperidol | 0.03 | + 9 ± 9 |
| (n=5) | 0.1 | − 36 ± 18 |
| | 0.3 | − 96 ± 1 |
| Metoclopramide | 1.0 | − 24 ± 13 |
| (n=5) | 3.0 | − 86 ± 2 |
| | 10 | − 99 ± 1 |
| Clozapine | 1.0 | − 22 ± 7 |
| (n=5) | 3.0 | − 52 ± 8 |
| | 10 | − 87 ± 12 |
| Clonidine | 0.01 | − 9 ± 17 |
| (n=6) | 0.03 | − 65 ± 5 |
| | 0.1 | − 91 ± 3 |
| Prazosin | 0.1 | + 3 ± 10 |
| (n=6) | 0.3 | − 46 ± 13 |
| | 1.0 | − 32 ± 9 |
| | 3.0 | − 91 ± 2 |

*All drug effects were significantly dose-related as indicated by regression analysis. Data indicate drug effects 30 min after treatment. Drug effects at 210 min after treatment did not exceed these at 30 min, and were usually smaller (data not shown).

not wholly attributable to the hypotensive "first-dose" phenomenon, which shows rapid tolerance [5]. In rats from the same stock as those used in the present experiments (i.e., normotensive Fischer), no tolerance was found over 2 days of treatment to the heart rate elevations and blood pressure changes produced by prazosin (Donald Miller, personal communication). To our knowledge, prazosin's effects on self-stimulation have not been previously described.

The ability of clonidine to alter ON latencies selectively [12] was confirmed. It was of interest that clonidine's effects on ON latencies appeared relatively more selective than those of the other drugs tested, although a high dose did elevate OFF latencies significantly above baseline. Clonidine's effects on self-stimulation have been attributed to its agonist activity on noradrenergic autoreceptors [13].

Interposition of a hurdle in the center of the apparatus did not alter ON and OFF latencies differentially, indicating that the ON latency is not necessarily more sensitive to an increase in physical difficulty of responding. At an apparently optimal dose, the muscle relaxant, methocarbamol, caused a relatively small elevation of ON latency by comparison with clonidine, clozapine, metoclopramide, haloperidol and prazosin. This increase in ON latency was, however, significant by comparison with baseline, and it significantly exceeded the increase in the OFF latency at this dose. These results lend limited credence to the criticism [9] that ON latencies may be relatively more susceptible than OFF latencies to some types of drug-induced performance disruption. Thus, a slightly greater increase in ON than in OFF latencies may not necessarily exclude a possible contribution of drug-induced muscle relaxation, contrary to an earlier suggestion from this laboratory [15].

The present results show that the bar-pressing rate measure yields results paralleling those from the ON latency in the shuttlebox procedure. An orderly proportionality of effective doses in the two procedures was observed for the six drugs that were examined. However, the shuttlebox procedure has greater versatility in the evaluation of experimental drugs than does the bar-pressing measure. A much larger effect on ON latencies than on OFF latencies may suggest relative selectivity for the "reward" component of stimulation, as indicated (for example) by the contrast between clonidine and methocarbamol. Moreover, anxiolytic activity is reflected by a selective increase in the OFF latency and the shuttlebox test may potentially discriminate among subclasses of anxiolytic drugs [11]. In addition, certain stimulant drugs also yield distinctive profiles in this test, selectively reducing the ON latency [16]. The shuttlebox self-stimulation procedure therefore constitutes a valuable tool for assessing drug effects on brain reward and aversion mechanisms.

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