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Relative Effectiveness of Pimozide, Haloperidol and Trifluoperazine on Self-Stimulation Rate-Intensity Functions

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LYNCH, M. R. AND R. A. WISE. *Relative effectiveness of pimozide, haloperidol and trifluoperazine on self-stimulation rate-intensity functions.* PHARMACOL BIOCHEM BEHAV **23**(5) 777–780, 1985.—Rats were trained to lever press for 60 Hz sine wave stimulation of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus. Rate intensity functions were determined by stepping down the current intensity by 0.05 $\log_{10}$ units every five min. Haloperidol shifted the function approximately one step to the right at 0.08 mg/kg, with lower doses producing no effect. Pimozide shifted the curve in a dose-dependent manner over the range of 0.06 to 0.24 mg/kg; 0.12 mg/kg produced an approximate one-step shift. Trifluoperazine also produced dose related shifts to the right over the range of 0.08 to 0.32 mg/kg; this agent, unlike the others, appeared to shift the curve down as well as to the right. Less than a one-step shift was produced by 0.08 mg/kg; more than a one-step shift was produced by 0.16 mg/kg. On a molar basis, the order of potency for displacing the curves to the right was haloperidol, pimozide and trifluoperazine. While this order of potency is not predicted from simple *in vitro* binding affinities for the D_2 receptor, this may be due to differences in the penetration of these agents to central D_2 receptors *in vivo*.

Brain stimulation reward Intracranial self-stimulation Neuroleptics Haloperidol Pimozide Trifluoperazine

NEUROLEPTIC drugs generally decrease behavioral output in a variety of situations [4, 13, 22, 23, 27, 30, 33, 34]. It is currently believed that neuroleptics have two relatively independent actions which result in decreases in behavior; neuroleptics are thought to alter both the motoric capacity of the animal and the motivational impact of rewarding stimuli which normally elicit behavioral responses [4, 10, 16, 20, 25, 26, 29]. Pimozide, an agent relatively selective for the D_2 dopamine receptor [5,28], produces clear and potent reductions in reward impact with relatively little impairment of motoric capability [2, 14–18, 26, 35]. Alpha-flupenthixol, an agent relatively selective for the D_1 dopamine receptor [28], is suggested to impair motoric capacity significantly without having great potency in attenuating reward [11]. Thus one possibility is that different dopamine receptors are associated with the reward-attenuating and the motor-impairing actions of neuroleptics. This view would fit with the fact that affinity for the D_2 receptor correlates well with ability of various neuroleptics to attenuate reward, whereas affinities for the D_1, D_3, alpha adrenergic, and serotonin S_1 and S_2 receptors do not [19].

To further explore the possibility that D_1 and D_2 receptor actions differentially mediate motor incapacitating and reward attenuating actions of neuroleptics, three neuroleptics with different affinities for these receptors were tested in a paradigm which allows some degree of dissociation between reward-attenuating and motor-incapacitating drug effects. This paradigm is a variation on the curve-shift paradigm of Edmonds and Gallistel [8]. In the curve-shift paradigm animals are tested at a number of currents or frequencies; the function relating response rate to stimulation intensity or frequency is an ogive. Two kinds of shift in this function have been produced with various reward-attenuating and motor-incapacitating treatments: a shift to the right and a shift down, respectively [8]. A simple shift down in the curve involves no change in the threshold level of frequency or intensity necessary to produce minimal responding but involves a decrease in the asymptotic maximal response rate. While one cannot infer conclusively that an animal is unable to respond faster than the asymptotic maximum observed, this is the usual interpretation of a decreased asymptote. It is reasoned that if the animals were capable of responding faster, then higher currents or frequencies, associated with stronger rewarding impact [21], should motivate them to do so. A simple shift to the right in the curve involves no change in the demonstrated response capability of the animal, but indicates that increased motivational impact is necessary to produce normal responding (regardless of whether the response demand is maximal or minimal).

The neuroleptics tested were pimozide, haloperidol and

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trifluoperazine. These agents are listed in order of affinity for the D_2 receptor; they have the reverse order of potency for the D_1 receptor [5, 6, 28]. If an action at the D_2 receptor were responsible for the reward-attenuating actions of these agents (and if the concentration of drug reaching the receptor were directly proportional to the dose administered), then pimozide should be most potent, and trifluoperazine least potent, at shifting the rate-intensity function to the right. If an action at the D_1 receptor were responsible for the motor-incapacitating actions, then trifluoperazine should be most potent, and pimozide least potent, at shifting the curves down.

METHOD

Subjects

Subjects were eighteen naive, male, Long Evans hooded rats. They were housed under a 12 hr light-dark cycle (8:00–20:00 hr), with food and water available ad lib. Their weights ranged from 300 to 450 g at the time of surgery.

Under sodium pentobarbital anesthesia (60 mg/kg), animals were stereotaxically implanted with bipolar stainless steel electrodes (Plastic Products, Model MS303), insulated with Formvar enamel except at the square cross-section of the tip. Electrodes were aimed at the medial forebrain bundle at the level of the lateral hypothalamus, using coordinates −0.8 mm A-P, 1.8 mm M-L and 8.2 mm ventral to dura (plane of deGroot [7]). The assembly was anchored by dental cement to stainless steel skull screws. An indifferent ground electrode was attached to one of the skull screws. At least five days of recovery followed before the initiation of behavioral testing.

Apparatus

Animals were trained and tested in 26×26 cm Plexiglas operant chambers, enclosed in wooden alcoves, with white noise and 15 W cue lights provided. Each lever press was rewarded with a 0.5 sec train of 60 Hz since wave stimulation. Stimulation intensity was adjusted for individual animals.

Drugs

All drugs were administered intraperitoneally in a volume of 1 ml/kg. Pimozide (Janssen Pharmaceuticals) was dissolved in warm 0.1 M tartaric acid. Haloperidol (McNeill Pharmaceuticals) was administered in the same vehicle, buffered to pH 5.6 with sodium hydroxide. Trifluoperazine HCl (Smith, Kline and French) was dissolved in distilled water. Doses refer to the salts.

Procedure

After recovery from surgery, rats were screened for intracranial self-stimulation on a CRF schedule of reinforcement. Bar pressing was shaped using the lowest current intensity that would maintain stable responding over 45–60 min sessions. After three days of training, a current intensity that would generate approximately 2000 responses per half hr period was determined for each animal. The animals were tested for three days in half-hour sessions at this fixed intensity. Then, rate intensity functions were determined for each rat: intensities were set to produce maximum (asymptotic) response rates for a 3–5 min warm-up period, followed by data collection for 3 min. Currents were then reduced in 0.05 log steps (the constant 0.05 was subtracted from the $\log_{10}$ of the preceding stimulation intensity to determine the log of the next intensity) for successive 5 min test periods. In each period the first two min were an "adjustment" period and data were collected for the last three min only. Testing was terminated when an intensity was reached that supported less than 10 bar presses per min.

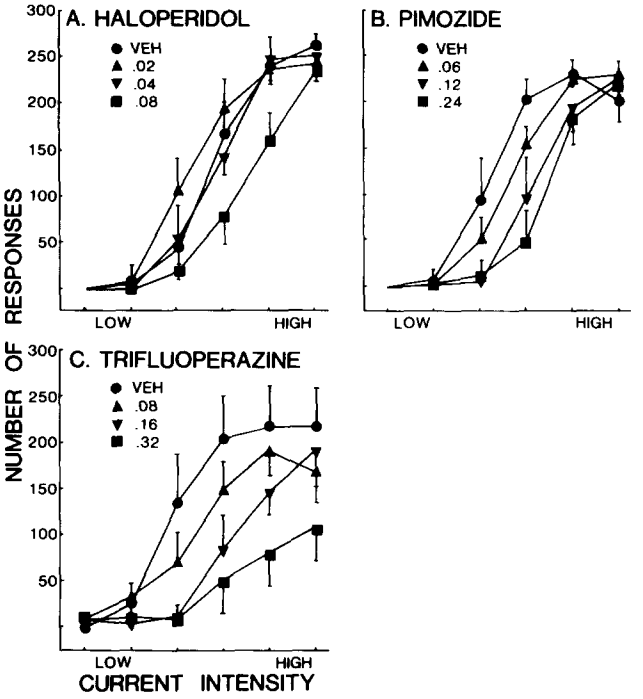

FIG. 1. Response rates (responses per three-minute test) at several stimulation intensities under (A) haloperidol, (B) pimozide, and (C) trifluoperazine. Stimulation intensity was varied in 0.05 $\log_{10}$ units; drug doses are given in mg/kg. Stimulation intensity varied between rats; the ranges for each group were 15 to 79 μA (haloperidol), 17.5 to 91 μA (pimozide) and 11 to 60 μA (trifluoperazine).

Stabilization testing continued in this manner for at least ten days, until both threshold values and response rates were stable (less than 10% variation across three consecutive days). Thresholds for the last few days prior to drug testing ranged from 17.5 to 60 μA (mean=36.8 μA). After stable performance was achieved rats were randomly assigned to separate drug groups (n=6 each). Each rat was tested under each of three doses of its assigned drug and drug vehicle; injections were given in a balanced order. Pimozide was given 5 hr 45 min before initiation of testing, as previous studies in this laboratory have determined a peak effect on BSR at 6 hr [3]. Times of peak action of haloperidol and trifluoperazine were found to be 1 hr and 3–5 hr, respectively; thus haloperidol was administered 45 min before testing and trifluoperazine was administered 2 hr 45 min before testing. Pimozide doses were 0.006, 0.12 and 0.24 mg/kg; haloperidol doses were 0.02, 0.04 and 0.08 mg/kg doses; and trifluoperazine doses were 0.08, 0.16 and 0.32 mg/kg. Drug testing was conducted every third day, with drug-free rate intensity functions determined on the two intervening days. One drug-free rate intensity function was also conducted 24 hr after the final drug test.

At the completion of testing rats were anesthetized with

chloral hydrate and perfused intracardially with saline, followed by a 10% formalin solution. The brains were removed and stored in formalin for at least 24 hr before being frozen and serially sectioned. Mounted 40 μm slices were stained with thionin for determination of electrode placements.

RESULTS AND DISCUSSION

Each of the three neuroleptics shifted the rate-intensity functions to the right; trifluoperazine shifted the functions down as well (Fig. 1). Only the highest dose of haloperidol (0.08 mg/kg) was effective; this dose shifted the rate intensity function approximately one 0.05 log unit to the right. The failure to shift the function to the right with lower doses was unexpected, since earlier reports indicate increased self-stimulation thresholds with doses as low as 0.006 mg/kg and decreased response rates with doses in the range of 0.01 to 0.1 mg/kg [9, 12, 24–26, 30]. If anything, 0.02 mg/kg seemed to improve performance in the present paradigm; such an effect might be expected from a dose which blocked autoreceptors but failed to block post-synaptic receptors [1]. Despite the fact that haloperidol seemed less potent in the present study than in earlier studies, haloperidol was still, in agreement with earlier studies [19], more potent than pimozide.

Pimozide caused a dose-orderly shift to the right across the range of doses tested. A shift of approximately one 0.05 log unit was produced by 0.12, but not by 0.06 mg/kg. Pimozide did not shift the rate-intensity function down, even at 0.24 mg/kg, in keeping with the findings of others [18,35]. At higher doses (0.5 mg/kg) and in slightly different paradigms [2] pimozide can cause shifts down as well as to the right [29]. Simple rate reductions are widely reported for this range of doses [12, 15, 24, 31, 32].

Trifluoperazine caused the strongest effects in the present study. A dose of 0.16 mg/kg produced more than a 0.05 log unit shift to the right of the rate-intensity function, while a dose of 0.08 mg/kg produced less than a 0.05 log unit shift. By interpolation a dose of 0.12 mg/kg of trifluoperazine would seem approximately equal in potency to the same dose of pimozide, producing a 0.05 log unit shift to the right. At 0.32 mg/kg trifluoperazine appeared to shift the function down as well as to the right, though the level of the asymptote cannot be clearly determined from the range of intensities tested, since the function seems still to be increasing at the highest intensity.

Doses that resulted in an approximate 0.05 log unit shift to the right in the rate-intensity functions were thus approximately 0.08 mg/kg for haloperidol and 0.12 mg/kg for both pimozide and trifluoperazine. When these doses are converted to μmoles/kg, (213 for haloperidol, 260 for pimozide and 271 for trifluoperazine), haloperidol remains most potent, with pimozide perhaps slightly more potent than trifluoperazine. This order of potency would not be predicted from the known orders of potency in either D_1 or D_2 receptor binding assays [5, 6, 28]. In D_1 binding trifluoperazine is most potent and pimozide least potent [28]; in D_2 binding pimozide is most potent and trifluoperazine least potent [5, 6, 28].

The present paradigm confirms the finding with other paradigms that haloperidol is more potent than pimozide in the attenuation of self-stimulation after intraperitoneal administration; inasmuch as affinity for the D_2 receptor does predict potency against self-stimulation for a wide range of neuroleptics, it is surprising that haloperidol, with lower affinity for the receptor, is consistently more potent than pimozide [19, 31, 32]. If affinity for the D_2 receptor predicts the effectiveness of most neuroleptics in this task, why does it not predict the relative effectiveness of these two agents?

The most fundamental consideration here is whether equal intraperitoneal doses of pimozide and haloperidol result in equal concentrations at the relevant receptor population. It is dose (concentration) at the receptor, not dose in the peritoneum, that should be plotted against the behavioral criterion in this study. Unfortunately, the concentration at the receptor is not known, and prediction from the injection dose may be subject to systematic error. The fact that pimozide does not have peak effectiveness until six hours after injection [3] suggests that pimozide has considerable difficulty in crossing the various diffusion barriers between the blood and the target of action. Haloperidol, which has peak behavioral action within the first hour, would seem to pass the diffusion barriers a great deal more readily. It may thus be that a greater fraction of the injected pimozide is metabolized before distribution is complete, and that a given dose of pimozide is less effective than a similar dose of haloperidol because it results in occupation of fewer D_2 receptors. Without direct measurements to determine whether equal injected doses result in different peak concentrations at the receptor, this seems the most attractive explanation of why affinity for the D_2 receptor predicts behavioral effectiveness generally for a range of neuroleptics but does not predict relative effectiveness for the specific case of haloperidol and pimozide.

It is clear that the affinity for the D_1 receptor does not predict effectiveness in this behavioral task. Trifluoperazine has the greatest affinity for the D_1 receptor, and since it has earlier peak action than pimozide it should reach the target receptors in higher concentration. Thus if the D_1 receptor were the target of action relevant to the present task trifluoperazine should have been clearly more potent than pimozide. On the other hand, haloperidol's greater potency than pimozide would be predicted from their relative potency at the D_1 receptor, but haloperidol should not have been more potent than trifluoperazine if the D_1 receptor were involved.

In summary, the relative effectiveness of intraperitoneal haloperidol, pimozide and trifluoperazine in attenuating the rewarding effects of hypothalamic brain stimulation was not predicted by the known affinity of these three agents, *in vitro*, for either the D_1 or the D_2 receptor. Differences in the time of peak action of these agents suggest, however, that equal intraperitoneal doses do not produce equal concentrations at the relevant central receptors. Affinity for the D_2 receptor *in vivo* may still predict the behavioral effectiveness of equal receptor concentrations of haloperidol, pimozide and trifluoperazine, as is suggested [19] when a wider range of neuroleptics is compared.

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