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A Comparison of Atropine, Benztropine and Diphenhydramine on the Reversal of Haloperidol Induced Suppression of Self-Stimulation

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CAREY, R. J. *A comparison of atropine, benztropine and diphenhydramine on the reversal of haloperidol induced suppression of self-stimulation.* PHARMAC. BIOCHEM. BEHAV. 17(4) 851–854, 1982.—Acute haloperidol administration (0.25, 0.5 mg/kg) produced a severe reduction in locomotor activity and operant responding for intracranial self-stimulation in rats. If the rats were also given 10 mg/kg of either diphenhydramine or benztropine, this behavioral inhibition was substantially reversed. Atropine (10 mg/kg), however, did not significantly alter the haloperidol inhibition. In a second study, the rats were tested 30 and 120 minutes after the haloperidol treatment (0.25 mg/kg). Again, diphenhydramine and benztropine but not atropine substantially reversed the haloperidol suppression after 30 minutes. When retested after 120 minutes, however, atropine and benztropine but not diphenhydramine partially reversed the haloperidol suppression. Thus, the present study provides support that diphenhydramine can be effective in reversing haloperidol induced suppression of self-stimulation but for a shorter duration than benztropine.

Atropine Benztropine Diphenhydramine Haloperidol Self-stimulation

BALANCE among neurotransmitters has increasingly become recognized as a necessary approach to understanding brain-behavior relations. This concept of balance has most extensively been applied to the extrapyramidal system where disturbances in dopaminergic-cholinergic balance has been linked to neuropathological and pharmacologically-induced movement dysfunction [2, 3, 11]. A fruitful application of this balance schema has been in the use of anticholinergics to relieve the Parkinsonian symptoms produced either by brain pathology or neuroleptics. Perhaps the most clinically efficacious anticholinergic drug, benztropine, however, has a more complex mode of action than blockade of cholinergic transmission. As pointed out by Klawans [11] the synthesis of benztropine by combining the anticholinergic, atropine and antihistaminic, diphenhydramine moieties was designed to take advantage of the antiparkinsonian properties of each of these drugs. While anticholinergics have remained important in the management of Parkinsonian symptoms less attention has been devoted to the antiparkinsonian efficacy of diphenhydramine. Coyle and Snyder [7], however, have demonstrated a mechanism which appears to account for the antiparkinsonian effect of diphenhydramine in that diphenhydramine as well as benztropine can strongly inhibit dopamine reuptake.

In an attempt to experimentally assess the antiparkinsonian efficacy of diphenhydramine the present study compares effectiveness of diphenhydramine, benztropine and atropine in reversing behavioral inhibition produced by haloperidol. The two behaviors studied were locomotor activity and self-stimulation. Several studies have shown that self-stimulation [17, 18, 19] can be virtually completely suppressed by 0.08 mg/kg haloperidol and that this inhibition can be reversed by anticholinergics as well as by a 10 mg/kg dose of benztropine. In the present study somewhat higher dose levels of haloperidol (0.25 and 0.5 mg/kg) were used in order to insure a severe motoric dysfunction. These dose levels were still within the range of a selective alteration in dopamine neurotransmission by haloperidol [9, 10, 15] and well below dose levels (5.0 mg/kg) with broad non-specific effects [1].

Subjects

Adult male Sprague-Dawley rats were used as subjects. Out of an original group of 23 implants, sixteen rats with stable self-stimulation were used. They were maintained in individual cages in a room with controlled temperature (22.0±1°C, humidity (60±5%), and light dark cycle (12:12 hr). Purina Rat Chow and tap water were always available.

Surgery

Rats were implanted with bipolar platinum electrodes

(Plastic Products Co., Roanoke, VA) insulated except for the cross-sectional area at the cut end [6]. Surgery was aseptic and performed with the rats under deep equithesin anesthesia (0.3 ml/100 g). Using A Kopf-stereotaxic instrument, the electrodes were placed in the brain and affixed to the skull with cranio-plastic cement. The electrodes were aimed at the lateral hypothalamic area using the following stereotaxic coordinates: 1.2 mm posterior to bregma, 1.5 mm lateral to the midline sinus, and 8.0 mm ventral to Dura. The incision bar was fixed 3.2 mm above the interaural line. Following surgery each rat was maintained on antibiotic therapy for several days.

Histology

Upon completion of behavioral testing, rats were sacrificed by ether anesthesia and then intracardially perfused with 0.9% saline followed by a 10% Formalin solution. Electrodes were removed using an electrode carrier while the skull was held in the stereotaxic instrument. After fixation in 10% Formalin for 10 days, an approximately 3 mm section containing the electrode tract was removed from each brain, embedded in paraffin, and from this tissue block 12 μ sections were cut, mounted and stained with luxol fast blue and cresyl violet [12]. The stained sections were examined microscopically and the electrode tips were localized to the lateral hypothalamic area between the fornix and the internal capsule.

Apparatus

Self-stimulation testing was conducted in three 26×24 cm operant chambers housed within sound attenuating enclosures (BRS/LVE No. 1417). Each chamber contained a response lever (Ralph Gerbrands Co., No. G6312) centered in a chamber wall 3.5 cm above the grid floor. A 28 V DC miniature lamp (No. 304) illuminated each chamber and white noise was broadcast whenever stimulation was available. Relay circuits with timers and digital counters programmed reinforcement, recorded bar presses and controlled session duration.

A Grass Brief-Pulse Biphasic Stimulation Model BPS1 was the source for the brain stimulation reinforcement. The stimulation consisted of pairs of biphasic rectangular pulses of 0.1 msec separated by a 0.1 msec interval between positive and negative pulses. The frequency of stimulation was 100 pulses/sec and the train duration was 0.2 sec. Current intensity was monitored continuously on each of three oscilloscopes (Textronix No. 502A) from the voltage drop access a 1-KΩ resistor in series with the animal. Each rat was connected to the stimulator through mercury-swivel commutators mounted above each chamber. In addition to self-stimulation, locomotor activity measurements were made. The activity measurements which preceded the self-stimulation tests were obtained using large photoactivity cages (BRS/LVE PAC-001). These black metal cylinders were 61 cm in diameter and 53 cm high with a metal grid floor. Six infrared photocells symmetrically spaced 2.5 cm above the floor detected movements. The photobeam interruptions were recorded on two digital counters with each counter recording from three photocells.

Four drugs were used in this experiment: haloperidol (M_rN-JR-1625 McNeil Laboratories), benztropine mesylate (Merck, Sharp and Dohme); atropine sulfate (Sigma Chemical Co.); and diphenhydramine HCl (Parke-Davis). The haloperidol was dissolved in warm lactic acid (1 g/1 ml) and diluted with distilled water to concentrations of 0.25 and 0.5 mg/cc. The benztropine, atropine and diphenhydramine were each dissolved in normal saline to a concentration of 10 mg/cc.

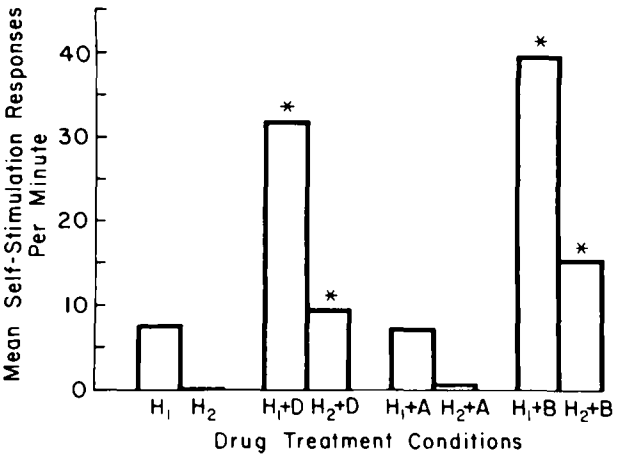

FIG. 1. Mean self-stimulation response rates following haloperidol alone (H_1=0.25 mg/kg, H_2=0.5 mg/kg) or in combination with either diphenhydramine (D) 10.0 mg/kg, atropine (A) 10.0 mg/kg, or benztropine (B) 10.0 mg/kg. *Denotes $p<0.05$ (t-test for difference scores).

Procedures

After a two-week postoperative recovery period, the rats were trained to bar press for the 0.2 sec of brain stimulation. After the response appeared well trained, the rats were given daily 15 minute sessions with brain stimulation available on a continuous reinforcement schedule until reliable response rates were established. Next, rate-intensity functions were determined for each rat. On the basis of the rate-intensity functions, each rat was tested at the lowest current intensity which generated the maximal response rate. Before the beginning of the drug testing, however, each rat was given 5 daily ten minute stimulation tests to establish reliable non-drug response performance [14]. The current intensities used in the predrug tests were then used throughout the course of the experiment.

Phase 1

The initial phase of this study compared the effects of 10 mg/kg doses of atropine, benztropine and diphenhydramine in reversing the suppression of self-stimulation produced by 0.25 and 0.5 mg/kg doses of haloperidol. The doses of benztropine, atropine and diphenhydramine were selected because of the efficacy of this dose of benztropine to reverse haloperidol-induced catalepsy and self-stimulation [6, 17, 19]. Eight rats were used and there were eight drug tests in all: two with just haloperidol and six combined haloperidol plus either atropine, benztropine or diphenhydramine. One drug test was given per week and each drug test day was preceded by a non-drug test day in order to maintain the non-drug baseline performance. Each rat received each drug treatment. Activity testing began 30 min after the drug injections. The activity test lasted 15 minutes and then was immediately followed by the 10 minute self-stimulation test.

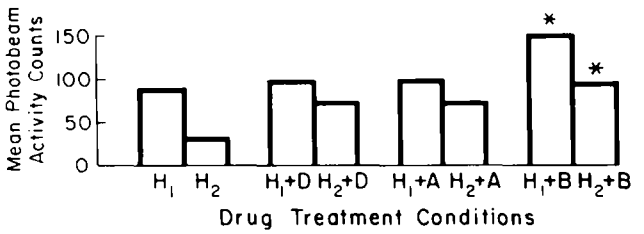

FIG. 2. Mean photobeam activity counts following haloperidol alone (H_1=0.25 mg/kg, H_2=0.5 mg/kg) or in combination with either diphenhydramine (D) 10.0 mg/kg, atropine (A) 10.0 mg/kg, or benztropine (B) 10.0 mg/kg. *Denotes $p<0.05$ (t-test for difference scores).

Phase 2

On the basis of some preliminary findings in which the reversal of haloperidol induced catalepsy by atropine and diphenhydramine appeared to depend on the time after injection, the second phase of this study was undertaken in which the testing was conducted 30 and 120 minutes after the drug treatments. In this part of the experiment only the 0.25 mg/kg dose of haloperidol was used. Eight different rats were used and all aspects of the procedure remained the same except that the test procedure which was conducted 30 minutes after the drug injections was repeated 120 minutes after the injection, namely, 15 minutes of activity testing followed by 10 minutes of self-stimulation testing.

RESULTS

The non-drug self-stimulation response rates varied from 45 to 110 responses per minute with a group mean response rate of 74 responses per minute. As can be seen in Fig. 1, haloperidol produced a severe dose dependent reduction in self-stimulation. Of importance to the present study, however, was the observation that benztropine and diphenhydramine produced comparable, albeit partial, reversals of the the haloperidol suppression of self-stimulation, whereas atropine did not have a statistically significant effect.

Figure 2 shows the effects of the drug treatments on locomotor activity. Again, haloperidol produced a dose dependent suppression of behavior in that activity was sharply reduced from the mean non-drug activity level of 310 counts. Benztropine, diphenhydramine and atropine has comparable but modest effects in reversing the haloperidol induced suppression of activity but only benztropine produced a statistically significant increase in activity level.

Figure 3 shows the effects of the atropine, benztropine and diphenhydramine on haloperidol suppression of self-stimulation at the two time intervals after the drug treatments. As can be seen in Fig. 3, haloperidol produced a virtual complete suppression of self-stimulation (non-drug baseline 68 responses per minute) which was substantially reversed by benztropine at both test intervals. The effects of atropine and diphenhydramine, however, proved to be time dependent. Diphenhydramine produced a statistically significant reversal of the haloperidol suppression early at the 30 minute post-injection test, whereas atropine only reversed the haloperidol suppression late at the 120 minute post-injection test. Figure 4 presents the effects of the drug treatments on locomotor activity. Haloperidol produced a severe reduction in activity from the non-drug baseline of 295

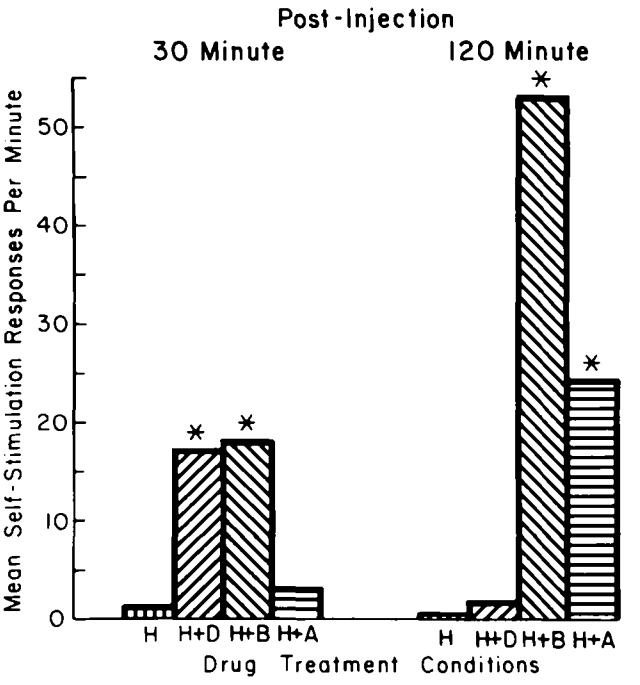

FIG. 3. Mean self-stimulation response rates at two time intervals after injection of haloperidol alone (H) 0.25 mg/kg or in combination with either diphenhydramine (D) 10.0 mg/kg, atropine (A) 10.0 mg/kg or benztropine (B) 10.0 mg/kg. *Denotes $p<0.05$ (t-test for difference scores).

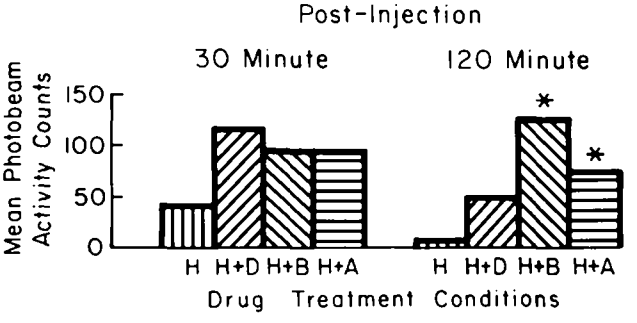

FIG. 4. Mean photobeam activity counts following haloperidol alone (H) 0.25 mg/kg or in combination with either diphenhydramine (D) 10.0 mg/kg, atropine (A) 10.0 mg/kg, or benztropine (B) 10.0 mg/kg. *Denotes $p<0.05$ (t-test for difference scores).

counts which was partially reversed by the three combined drug treatments. All three treatments produced overall but not statistically significant elevations in activity over the haloperidol level at the 30 minute test but atropine and benztropine elevated activity significantly ($p<0.05$) above the haloperidol level at the 120 minute test.

DISCUSSION

The results of the present study show that diphenhydramine can be effective in the reversal of neuroleptic induced suppression of self-stimulation. When tests were done within the first hour of drug administration diphenhydramine was of

comparable effectiveness to benztropine. Several hours after administration, however, diphenhydramine was no longer effective whereas benztropine continued to counteract the self-stimulation inhibition produced by haloperidol. Atropine, on the other hand, had generally the opposite effect of diphenhydramine in attenuating the self-stimulation deficit, namely, little effect early but a significant effect several hours after administration. Possibly, this apparent delayed effect of atropine reflected untoward effects of this rather high dose level of the anticholinergic which interfered with its facilitative influence initially, but, after several hours, the interfering effects abated permitting the facilitative influence of atropine to become manifest.

The finding that diphenhydramine can alleviate behavioral suppression induced by haloperidol complements a recent report [8] which indicates that diphenhydramine can be very effective in reducing extrapyramidal side effects induced by excessive haloperidol use in humans. Since several studies [4,16] have suggested that the use of anticholinergics to reverse neuroleptic induced extrapyramidal side effects may exacerbate the likelihood for tardive dyskinesia the apparent effectiveness of diphenhydramine in reversing neuroleptic parkinsonian symptoms suggests that this drug treatment may merit attention in the treatment of neuroleptic induced side effects.

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