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Possible Dissociation of Central Dopamine Receptor Antagonism and Cataleptic Behavior

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HRUSKA, R. E. *Possible dissociation of central dopamine receptor antagonism and cataleptic behavior.* PHARMACOL BIOCHEM BEHAV **23**(5) 789–795, 1985.— A new chemical, 2-[4-[4-(7,9-dioxo-6-thia-8-azaspiro[4.4] nonan-8-yl)-butyl]-1-piperazinyl]pyridine-3-carbonitrile hydrochloride (MJ-13980-1), referred to in this report as MJ-13980, displaced in vitro [^{3}H]spiperone binding, elevated serum prolactin concentrations, and decreased apomorphine-induced stereotyped behavior in male rats. These actions indicated that MJ-13980 acts as a dopamine (DA) receptor antagonist. However, MJ-13980 (10 mg/kg) elicited only a very small amount of cataleptic behavior and antagonized that produced by haloperidol (HAL), a classical DA receptor blocker and potent inducer of cataleptic behavior. At a lower dose, MJ-13980 (1.0 mg/kg) produced no cataleptic behavior but again decreased that produced by HAL. These results suggest that the antagonistic interaction of MJ-13980 at central DA receptors is not associated with the production of cataleptic behavior.

Cataleptic behavior Dopamine receptors Prolactin Stereotyped behavior Haloperidol MJ-13980-1

VARIOUS compounds of diverse chemical structures (e.g., butyrophenones, phenothiazines, and thioxanthines) have been shown to inhibit dopamine (DA) receptors [13], elevate serum prolactin concentrations [1], antagonize stereotyped behavior [10], and produce cataleptic behavior [10]. The only caveat was the demonstration that the compounds entered the central nervous system and reached the striatum, which is the anatomical location for the production of stereotyped [2, 4, 9, 14] and cataleptic [12] behaviors. Furthermore, the activity of these compounds to antagonize stereotyped behavior correlated well with their production of cataleptic behavior [10].

The compound MJ-13980 (chemically noted in the abstract) has been identified as having properties of a DA receptor antagonist [11]. Included in these observations are the displacement of [^{3}H]spiperone binding and the blockade of apomorphine-induced stereotyped behaviors. However, MJ-13980 was also described as lacking cataleptic-inducing properties and actually reversing the cataleptic behavior induced by a phenothiazine [11]. Therefore, the ability of this compound to induce cataleptic behavior and antagonize apomorphine-induced stereotyped behavior may not correlate. To describe more fully this novel effect on DA receptors, the activity of MJ-13980 was evaluated on pituitary DA receptors by measuring serum prolactin concentrations. Further, these biochemical and behavioral effects were compared to a classical DA receptor antagonist, the butyrophenone, haloperidol (HAL).

METHOD

Animals

Male, adult Sprague-Dawley rats, weighing 200–300 g, were obtained from Taconic Farms, Germantown, NY. Rats were injected IP with the appropriate drug or vehicle solution (saline) and observed for specific behaviors. After behavioral observation they were decapitated and the trunk bloods collected for prolactin measurements.

Prolactin Measurements

The trunk bloods were collected into 50 ml plastic centrifuge tubes and allowed to clot at room temperature. The samples were then centrifuged at 5000×g for 15 min in a refrigerated centrifuge at 10°C. Each serum was removed and stored at −80°C in plastic tubes. All samples were analyzed within 2 months. After thawing and mixing, the samples were centrifuged in a microcentrifuge (13,000×g) for 1.0 min before duplicate 0.1 ml aliquots of the supernatants were removed into glass test tubes.

Rat prolactin (rPRL) was measured by radioimmunoassay (RIA) using the standard rPRL (RP-2) and specific antibody (S-8) provided by the National Pituitary Agency. [^{125}I]rPRL was purchased from New England Nuclear. The assays were performed in a buffer (PRL buffer) containing 10 mM Na_2HPO_4/NaH_2PO_4 buffer (pH 7.6), 160 mM NaCl, 10 mM Na_2EDTA, 0.1% NaN_3, and 1% BSA (RIA grade). The antiserum was diluted 1:1000 in PRL buffer containing 1% normal rabbit serum (Gibco). Each tube (all determinations in duplicate) contained 0.2 ml PRL buffer, 0.1 ml rPRL standard or unknown serum, 0.1 ml [^{125}I]rPRL (20,000–30,000 cpm/tube), and 0.1 ml antiserum. The standard curves were prepared using serial dilutions of rPRL from 1–200 ng/ml. Unknown samples which contained very large amounts of rPRL were diluted 10-fold with PRL buffer before addition to the tubes. The tubes were vortexed,

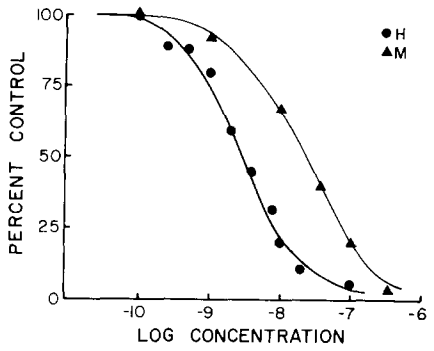

FIG. 1. In vitro inhibition of specific [^{3}H]spiperone binding by haloperidol (H) or MJ-13980 (M). Non-specific binding, defined by 1.0 μM d-butaclamol, was subtracted. Each point is the average of six values from two separate experiments.

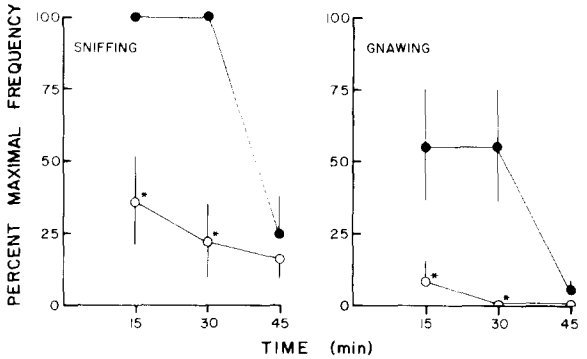

FIG. 2. The production of stereotyped behavior by APO (2 mg/kg) is inhibited by MJ-13980 (10 mg/kg). Two behaviors indicative of stereotyped behavior were recorded, namely sniffing and gnawing. Each point is the mean±SEM of the scores of six rats. (No SEM bar indicates that the SEM is within the size of the symbol.) Rats were injected with either saline (closed symbols) or MJ-13980 (open symbols) and 15 min later all rats received an IP injection of APO. The time indicated is after the APO injection. *Significantly different ($p<0.05$) compared to the saline + APO treated group.

covered, and incubated at room temperature for 48 hr. Subsequently, 0.1 ml of goat anti-rabbit gamma-globulin (Gibco), diluted 1:4 in PRL buffer, was added to each tube. The tubes were vortexed, covered, and allowed to incubate at room temperature for 24 hr. Finally, distilled water (2 ml) was added and the tubes centrifuged at 1600×g for 45 min. The supernatant was decanted, the tubes dried, and the radioactivity quantitated with a gamma counter. Unknown samples were compared to standard curves, fitted by least-squares linear regression analyses.

In Vitro Receptor Binding

The potency of MJ-13980 in vitro on the displacement of specific [^{3}H]spiperone binding to DA receptors was performed as described previously [7]. Briefly, the rat brain was removed, the striatal tissue was dissected and homogenized in 100 mM Na_2KPO_4 buffer at pH 7.4, and the homogenate was washed twice by centrifugation at 40,000×g for 10 min. MJ-13980 or HAL was dissolved in distilled water and added to sets of duplicate tubes at final concentrations ranging from 1×10^{-5} to 1×10^{-10} M. Aliquots of the homogenate were incubated with [^{3}H]spiperone (65±3 pM) in 10 ml of buffer for 30 min at 37°C with or without MJ-13980 or HAL. One set of tubes received d-butaclamol (1.0 μM) in order to define non-specific binding. The solutions were filtered through GF/B filters and the tubes and filters were washed with ice-cold buffer. The filters were placed into vials with scintillation fluor and the radioactivity quantitated by standard procedures. The concentrations required to inhibit 50% of the specific binding (IC_{50} values) were calculated from least squares linear regression analyses of Hill [6] plots.

Stereotyped Behavior to Apomorphine

Stereotyped behavior was induced by the IP injection of apomorphine hydrochloride (APO) at a dose of 2 mg/kg, as described previously [8]. At 15 min prior to the APO injection the rats were injected IP with either saline (1 ml/kg) or MJ-13980 (10 mg/kg in a volume of 1 ml/kg).

In this experiment only sniffing and gnawing were used as indexes of stereotyped behavior. Every 15 min after APO injection each rat was rated for 6 intervals of 10 sec each (total 60 sec) for the exhibition of sniffing and/or gnawing behaviors. Each rat could receive a maximal frequency score of 6 for sniffing and 6 for gnawing during every rating period, i.e., every 15 min. Rats were rated through 45 min after APO injection. All ratings were converted to a percent of the maximal frequency. At 105 min after APO injection (120 min after MJ-13980) the rats were decapitated and their trunk bloods collected for rPRL measurements.

Cataleptic Behavior Measurements

Cataleptic behavior was measured as reported previously [3]. A shortened description follows. The rats were timed by stopwatch for three specific behaviors. First, akinesia was measured after placing the rat gently on an open, flat surface. Akinesia was present until the rat moved three of its four paws, with a maximal duration of 60 sec allowed. Second, hanging (or leaning) was measured after placing the rat's head and forelimbs over a bar 4.5 mm in diameter and 10 cm above the floor of the device and its hindpaws on the floor of the device. In this manner the rat was in contact with the bar by its armpits. Hanging was present until the rat moved off the bar or moved one hindpaw to the bar, with a maximal duration of 60 sec allowed. Third, clinging was measured after placing the rat's forepaws on a bar 4.5 mm in diameter and 18 cm above the floor of the device and its hindpaws on a similar bar 10 cm above the floor of the device. The rat had to cling to the bars to prevent falling off the bars. Clinging was present until the rat moved one of its paws to the other bar or fell from the bars, with a maximal duration of 120 sec allowed. All three measurements were made on each rat at 30 min intervals and were expressed as a percent of the maximal responses allowed.

Besides these three individual components of cataleptic behavior (i.e., akinesia, hanging, and clinging), a composite score was obtained at each time interval by averaging the percent scores for each rat on the three individual measurements. The composite score was then evaluated separately. The use of the composite score reduced the variability due to an unusual response in only one parameter, and also reduced the variability of the scores between rats.

TABLE 1

SERUM RAT PROLACTIN LEVELS

Initial Treatment		Second Treatment			Measurements		
Drug	Dose (mg/kg)	Drug	Dose (mg/kg)	Time* (min)	Time* (min)	rPRL (ng/ml)	(N)
Experiment 1							
Saline	—	APO	2	15	120	67.0 ± 17.3	(6)
MJ-13980	10	APO	2	15	120	139.9 ± 17.3†	(6)
Experiment 2							
Saline	—	HAL	0.5	30	90	207.5 ± 38.4	(9)
MJ-13980	10	HAL	0.5	30	90	198.0 ± 20.2	(9)
Experiment 3							
Saline	—	Saline	—	30	120	15.7 ± 3.5	(7)
Saline	—	MJ-13980	10	30	120	102.1 ± 15.2‡	(7)
HAL	0.5	Saline	—	30	120	138.4 ± 4.2‡	(7)
HAL	0.5	MJ-13980	10	30	120	191.3 ± 21.5‡§¶	(7)
Experiment 4							
Saline	—	Saline	—	30	120	18.3 ± 3.8	(7)
Saline	—	MJ-13980	1	30	120	32.4 ± 5.0‡	(7)
HAL	0.5	Saline	—	30	120	109.2 ± 14.3‡	(7)
HAL	0.5	MJ-13980	1	30	120	137.2 ± 12.3‡§	(7)

Each value is the mean ± SEM. APO=apomorphine, HAL=haloperidol, rPRL=rat prolactin. *Time after initial treatment. †Significantly different ($p<0.025$) compared to Saline + APO treatment group. ‡Significantly different ($p<0.05$) compared to respective Saline + Saline treatment group. §Significantly different ($p<0.005$) compared to respective Saline + MJ-13980 treatment group. ¶Significantly different ($p<0.05$) compared to HAL + Saline treatment group.

Experiments on cataleptic behavior were performed in two ways and comparisons were made to the HAL treatment. In the first series of experiments, rats were rated for cataleptic behavior and then immediately injected with either saline or MJ-13980 (10 mg/kg, IP). The rating of cataleptic behavior at 30 min after injection was followed immediately by the injection of HAL (0.5 mg/kg, IP) to all rats. Ratings were made again at 60 and 90 min after the saline or MJ-13980 injection. After the 90 min ratings the rats were decapitated and the trunk bloods collected for serum rPRL measurements.

In the second series of experiments on cataleptic behavior, rats were rated and then immediately injected with either saline or HAL (0.5 mg/kg, IP). Rats were rated at 30 min and then immediately injected with either saline or MJ-13980 (1 or 10 mg/kg, IP). Ratings were made again at 60, 90, and 120 min after the HAL injection. After the last ratings the rats were decapitated and the trunk bloods collected for serum rPRL measurements.

Statistical Evaluations

Statistical comparisons were made by analyses of variance [15]. For the stereotyped behavior experiments the analyses of variance were preceded by the arcsine transformation of the data [17]. In all cases, subsequent individual comparisons were made using the analyses of variance for only the two groups being compared [15].

RESULTS

The chemical MJ-13980 did inhibit the in vitro binding of [^{3}H]spiperone to striatal DA receptors. The IC_{50} value for MJ-13980 was 25.0±1.3 nM (Fig. 1). In comparison, the IC_{50} value for HAL was 3.09±0.72 nM (Fig. 1). The slopes of the inhibition lines in the Hill [6] plots were 0.920±0.046 for MJ-13980 and 0.959±0.070 for HAL with very good linear regression correlation coefficients (values > 0.984). Approximately 90% of the binding was inhibited by each compound, which is the same percentage as that inhibited by d-butaclamol, a stereospecific inhibitor of striatal DA receptors. From the IC_{50} values, HAL is about eightfold more potent than MJ-13980.

The effects of MJ-13980 on serum rPRL concentrations and on behavioral changes were measured in the same groups of rats. Rats were treated with MJ-13980 (10 mg/kg), followed 15 min later by APO (2 mg/kg), and compared to rats treated with saline and APO. The rats treated with MJ-13980 displayed significantly less stereotyped behavior (sniffing and gnawing) at 15 and 30 min after the APO injection than the group pretreated with saline (Fig. 2). Gnawing behavior in particular was completely blocked. By 45 min the action of APO had ended and both measurements of stereotyped behavior were at equivalent low levels. At 120 min after the MJ-13980 or saline injection, the serum rPRL concentrations were somewhat higher in the saline plus APO treated rats than those in the untreated and unstressed rats (Table 1; compare saline groups of experiments 1 and 3). The treatment with MJ-13980 did elevate the serum rPRL concentrations by about twofold (Table 1, experiment 1).

In the first experiment on cataleptic behavior, the rats received an initial treatment with MJ-13980 or saline. At 30 min after injection, MJ-13980 (10 mg/kg) produced a small but significant increase in cataleptic behavior, as measured on all three of the components and the composite score (Fig. 3). Administration of HAL (0.5 mg/kg), to the rats pretreated with saline, produced marked cataleptic behavior at 60 and

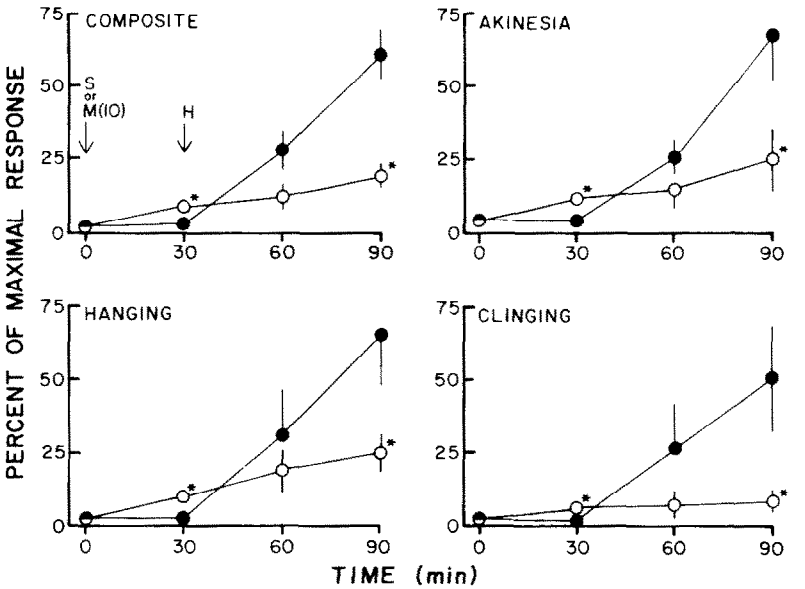

FIG. 3. The production of cataleptic behavior by haloperidol (0.5 mg/kg) is decreased by MJ-13980 (10 mg/kg). Three components of cataleptic behavior (akinesia, hanging, and clinging) were recorded and a composite score derived from them. Each point is the mean±SEM of the scores of nine rats. (No SEM bar indicates that the SEM is within the size of the symbol.) Rats were injected with either saline (S)(closed symbols) or MJ-13980 at 10 mg/kg (M(10))(open symbols) and 30 min later all rats received haloperidol (H). *Significantly different ($p<0.05$) compared to the saline + haloperidol treated group.

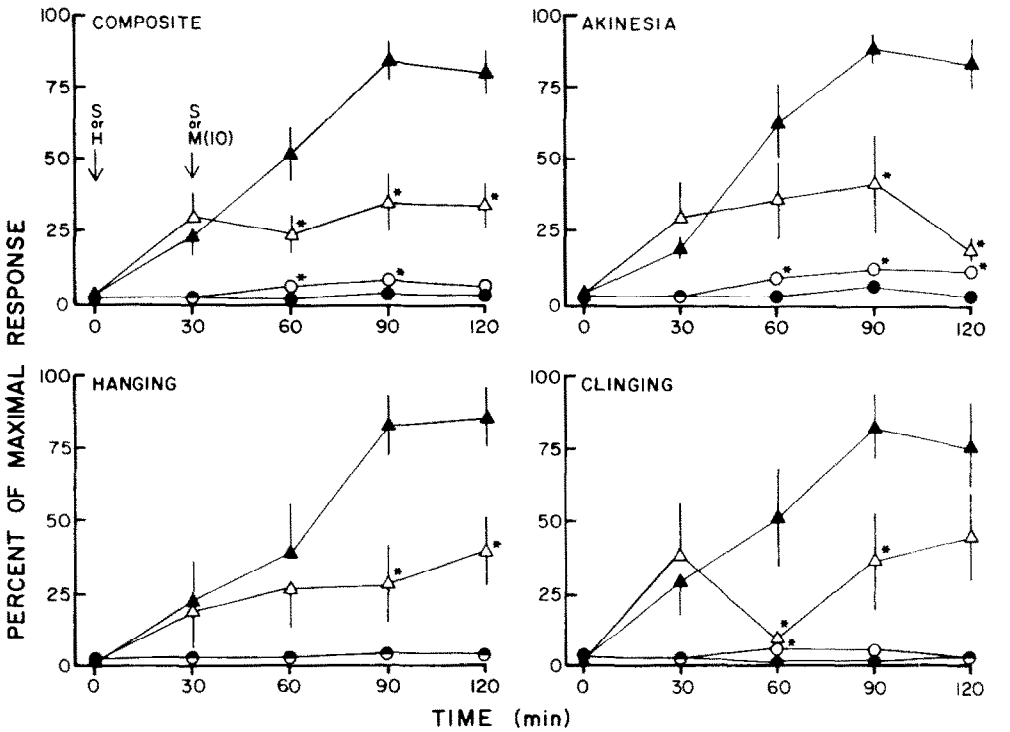

FIG. 4. The production of cataleptic behavior by haloperidol (0.5 mg/kg) is reversed by MJ-13980 (10 mg/kg). Three components of cataleptic behavior (akinesia, hanging, and clinging) were recorded and a composite score derived from them. Each point is the mean±SEM of the scores of seven rats. (No SEM bar indicates that the SEM is within the size of the symbol.) Rats were injected with either saline (S) or haloperidol (H) and 30 min later with either saline (S) or MJ-13980 at 10 mg/kg (M(10)). Closed circles=S + S; open circles=S + M(10); closed triangles=H + S; and open triangles=H + M(10). *Significantly different ($p<0.05$) for either the open circles compared to the closed circles (S +M(10) compared to S + S) or the open triangles compared to the closed triangles (H + M(10) compared to H + S).

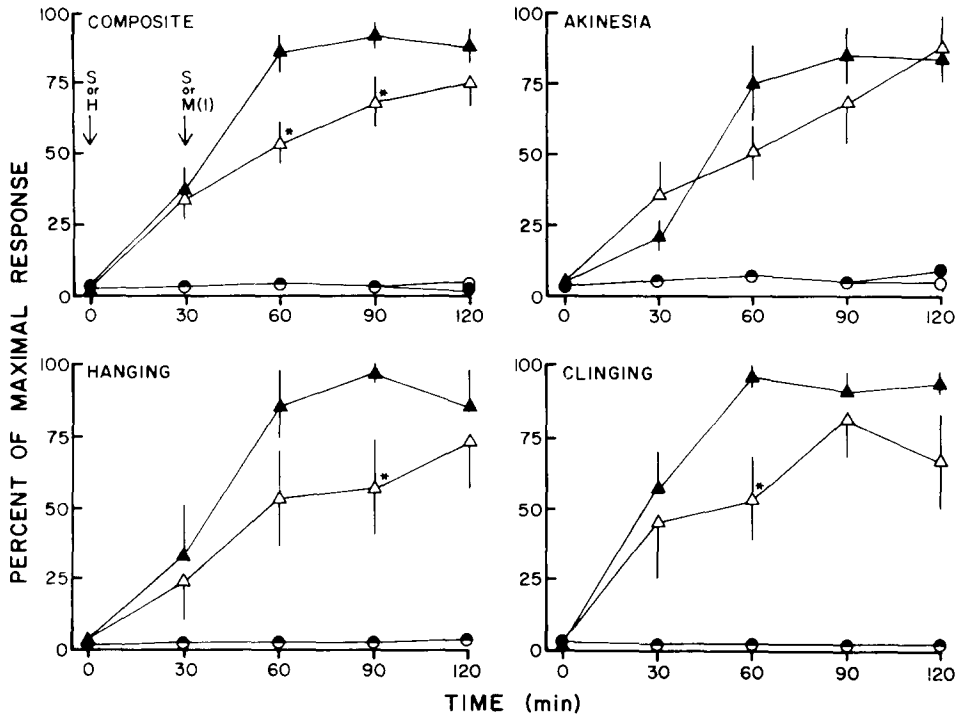

FIG. 5. The production of cataleptic behavior by haloperidol (0.5 mg/kg) is reversed by MJ-13980 (1.0 mg/kg). Three components of cataleptic behavior (akinesia, hanging, and clinging) were recorded and a composite score derived from them. Each point is the mean±SEM of the scores of seven rats. (No SEM bar indicates that the SEM is within the size of the symbol.) Rats were injected with either saline (S) or haloperidol (H) and 30 min later with either saline (S) or MJ-13980 at 1.0 mg/kg (M(1)). Closed circles=S + S; open circles=S + M(1); closed triangles=H + S; and open triangles=H +M(1). *Significantly different ($p<0.05$) for the open triangles compared to the closed triangles (H + M(1) compared to H +S).

90 min after saline injection (30 and 60 min after HAL). However, 90 min after the administration of HAL there was significantly less cataleptic behavior in the rats pretreated with MJ-13980 than in those pretreated with saline. This was observed on all three of the components and the composite score of the cataleptic behavior (Fig. 3). The decrease was more than 60%. The serum rPRL concentrations at 90 min after the initial treatment (60 min after HAL) were equivalent in the saline plus HAL and the MJ-13980 plus HAL groups of rats, and the concentrations were very high, i.e., 200 ng/ml (Table 1).

To better define the effects of MJ-13980 and its interaction with HAL, additional experiments were performed with the HAL treatment preceeding the MJ-13980 treatment. At 30 min after the injection of HAL (0.5 mg/kg) there was a large increase in cataleptic behavior, as measured on all three components and the composite score (Figs. 4 and 5). After this rating, the rats were injected immediately with either saline or MJ-13980 (1 or 10 mg/kg). Administration of MJ-13980 at 10 mg/kg significantly increased cataleptic behavior by about twofold in saline pretreated rats at 60 and 90 min after the saline injection (30 and 60 min after MJ-13980)(Fig. 4). This was observed primarily in the akinesia measurement but was also present in the composite score. Administration of MJ-13980 at 1.0 mg/kg produced no cataleptic behavior (indistinguishable from saline treatments) (Fig. 5). The cataleptic behavior produced by HAL pretreatment, followed by a saline injection, increased with each measurement and remained at high levels throughout these experimental protocols (Figs. 4 and 5). Administration of MJ-13980 at 10 mg/kg, despite producing some cataleptic behavior by itself, significantly decreased the HAL-induced cataleptic behavior to less than 50% of that for HAL alone at the 60, 90, and 120 min ratings (Fig. 4). This was demonstrated most clearly in the analysis of the composite score. Administration of MJ-13980 at 1.0 mg/kg also interacted significantly with the HAL-induced cataleptic behavior at 60 and 90 min, decreasing the cataleptic behavior more than 30% (Fig. 5). Again, this was most clearly demonstrated in the analysis of the composite score.

The serum rPRL concentrations were measured at 120 min, immediately after the last rating. The saline treated groups of rats had normal serum rPRL concentrations. Administration of HAL (0.5 mg/kg) significantly increased the serum rPRL concentrations over sixfold to more than 110 ng/ml (Table 1, experiments 3 and 4). This is lower than the serum rPRL concentrations obtained at 60 min after HAL in the previous experiment (Table 1, experiment 2) and probably reflects a decrease in the maximal serum concentrations from 60 to 120 min after the HAL injection. Administration of MJ-13980 at 1.0 mg/kg significantly increased by about twofold the serum rPRL concentrations, and at 10 mg/kg significantly increased the concentrations more than sixfold to about 100 ng/ml (Table 1). The interaction of HAL and a low dose of MJ-13980 (1.0 mg/kg) suggested a possible additive effect, but this was not significant. The effect of HAL

and a high dose of MJ-13980 (10 mg/kg) on serum rPRL concentrations was additive and significantly greater than for either treatment alone, reaching about 190 ng/ml.

DISCUSSION

MJ-13980 clearly fits several of the criteria for a DA receptor antagonist. First, it interacts at the striatal DA receptors as measured in vitro by the inhibition of [^{3}H]spiperone binding. It is fairly potent, having an IC_{50} value of 25.0 nM, compared to HAL with an IC_{50} value of 3.09 nM. While MJ-13980 is about eightfold less potent than HAL, it is still well within the range of other useful DA receptor drugs employed as antipsychotics [13]. The slopes of the Hill [6] plots and the good correlation coefficients indicate a single population of non-interactive receptor sites are displaced by MJ-13980 and HAL.

Second, similar to other DA receptor antagonists [1], MJ-13980 inhibits the pituitary DA receptors, resulting in an increase in serum rPRL concentrations. MJ-13980 is quite potent increasing concentrations twofold at 1.0 mg/kg and sixfold at 10 mg/kg, this later increase being approximately the same as that produced by HAL at 0.5 mg/kg.

Third, in accord with other DA receptor antagonists [10], MJ-13980 blocks the stereotyped behavior produced by APO. Since the production of stereotyped behavior by APO occurs following stimulation of striatal DA receptors [2, 4, 9, 14], MJ-13980 must penetrate into the central nervous system and reach the striatum.

Another criteria for classical DA receptor antagonists is the production of marked cataleptic behavior [10] by a specific action on striatal DA receptors [12]. However, MJ-13980 fails to produce cataleptic behavior when used at a low dose (1.0 mg/kg) and produces only a small amount of cataleptic behavior, compared to HAL, when used at a high dose (10 mg/kg). It should be noted that the amount of cataleptic behavior produced by MJ-13980, even at 10 mg/kg, is less than 10% of the maximal response. This low score is observed in the present experiments despite the fact that the tests used to define cataleptic behavior are exquisitely sensitive to DA receptor antagonists. For instance, this testing procedure has been used to demonstrate a prominent cataleptic behavior (40% of the maximal response) to a very low dose of HAL (0.1 mg/kg) [3]. Therefore, the sensitivity of these tests indicate that MJ-13980 is very weak in its ability to produce cataleptic behavior. This low potency correlates poorly with its potency on stereotyped behavior, in particular since both behaviors are mediated by striatal DA receptors.

Furthermore, most remarkably, MJ-13980 decreases in a dose-dependent manner the cataleptic behavior produced by HAL, whether MJ-13980 is administered before or after HAL. The interpretation of these actions on cataleptic behavior precludes the identification of MJ-13980 as a classical DA receptor antagonist. The action of DA receptor antagonists should be additive as, for instance, they are on serum rPRL concentrations (i.e., pituitary DA receptors). In contrast, the effects of MJ-13980 and HAL on the striatal DA receptors associated with the production of cataleptic behavior are not additive, in fact, they are opposing. Therefore, MJ-13980 produces an unusual interaction which suggests that striatal DA receptor antagonism may not be associated directly with the production of cataleptic behavior, and may even be associated with the prevention of cataleptic behavior.

Two neurochemical explanations for this dissociation are possible. First, it is known that GABA antagonists antagonize the cataleptic behavior produced by HAL [16]. If MJ-13980, besides its activity as a DA receptor antagonist, also possesses properties of a GABA antagonist, it could reduce cataleptic behavior. However, GABA antagonists are proconvulsant and MJ-13980 has not been observed in these experiments or reported in the literature to induce convulsive activity. Therefore, an interaction of MJ-13980 with GABA is probably not involved.

Second, cholinergic antagonists are reported to reverse the cataleptic behavior produced by HAL [5]. MJ-13980 has been tested in vitro and in vivo for anticholinergic activity and found to be inactive [11]. Therefore, the inhibition of cataleptic behavior by MJ-13980 is not related to an interaction at cholinergic receptors. Additionally, the routine observation of the rats after MJ-13980 administration was not associated with any unusual or abnormal behaviors which might result from interactions with other neurotransmitters. These reports and observations suggest that MJ-13980 does not possess the additional properties of a GABA or a cholinergic antagonist. However, similar neurochemical interactions on other systems cannot be eliminated at the present time.

An interesting hypothesis is that MJ-13980 acts at the pituitary DA receptors differently than it acts at those in the striatum. In the anterior pituitary a classical inhibition at the DA receptors would be additive to that of HAL. Hence, the independent effects to increase serum rPRL concentrations would be summed. In the striatum an unusual inhibition would be opposing to that of HAL. Action at this striatal site would produce neither agonist nor antagonist effects, or only very weak effects, but would block potently the ability of other compounds, whether DA agonists or antagonists, to act at these DA receptors, thereby blocking the production of both stereotyped and cataleptic behaviors. This dual inhibition would require that MJ-13980 acts at a modulatory or regulatory site associated with the striatal DA receptors. Such an interaction suggests that MJ-13980 may possess a unique pharmacological profile, one which warrants further characterization.

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