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Neuroleptic-Induced Catalepsy: A D_2 Blockade Phenomenon?

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KLEMM, W. R. *Neuroleptic-induced catalepsy: A D_2 blockade phenomenon?* PHARMACOL BIOCHEM BEHAV **23**(6) 911–915, 1985.—Typical neuroleptics, such as haloperidol, are cataleptogenic. But since such drugs block both D_1 and D_2 receptors, it is not clear if there is a differential receptor role in catalepsy. To test this issue in a mouse model of catalepsy, these experiments tested molindone, a D_2-blocking neuroleptic with almost no ability to block D_1 receptors. If D_1 receptor blockade is necessary for catalepsy, molindone should not cause catalepsy. But molindone *was* cataleptogenic, albeit less potent than haloperidol. There was also a "training effect" with haloperidol, but not saline or molindone, in that the catalepsy produced by 5 mg/kg of haloperidol was much greater when tests were performed repeatedly at short intervals after injection. Concurrent administration of apomorphine (4 or 8 mg/kg) markedly potentiated haloperidol catalepsy, but had no effect on molindone catalepsy. Such results are not readily interpretable solely in terms of current concepts of D_1 and D_2 receptors.

Dopamine Molindone Haloperidol Catalepsy Apomorphine Receptors

ONE strategy for elucidating the neuropharmacological mechanisms of movement and locomotor activity is to focus on immobility states, such as catalepsy. A common operational definition of catalepsy is that it is an akinetic state in which animals have sufficient muscular tone to sustain awkward positions. The immobility is more than a simple failure to initiate movement because animals will remain immobile for long periods in awkward postures. Catalepsy is readily produced and quantified by dopamine receptor blockers, such as haloperidol; in fact catalepsy is a cardinal sign of neuroleptic action in rodents.

A common view of dopamine receptors is that there are two functionally distinct types, D_1 and D_2 [6, 7, 10]. The primary distinctions are that blockade of D_1 receptors inhibits adenylate cyclase activity, while blockade of D_2 receptors reduces striatal acetylcholine levels by promoting its release [23]. Haloperidol-induced catalepsy could result from blockade of either or both receptor types, because it blocks both, although haloperidol has nanomolar potency for D_2 receptors and micromolar potency for D_1 receptors [7, 10, 23]. Thus, it is tempting to speculate that catalepsy is caused by blockade of D_2 receptors.

This present research explored the cataleptogenic effects of molindone, a selective D_2 blocker [7, 9, 10, 23] that is sometimes used to treat schizophrenia. If D_2 receptor blockade is necessary for catalepsy, molindone's selective D_2 blockade should make it very cataleptogenic, perhaps more so than haloperidol. Previous studies with another D_2 blocker, sulpiride, suggest that D_2 blockade causes little or no catalepsy [8,15].

It also seemed important to compare molindone and haloperidol for their cataleptogenic effects when they are combined with apomorphine. We have recently shown that apomorphine, given at the same time as haloperidol, markedly potentiated the degree of catalepsy caused by haloperidol alone [13]. If differential receptor action is involved in catalepsy, apomorphine should be tested in combination with a selective D_2 blocker such as molindone.

METHOD

Subjects

Experiments were conducted on male outbred Swiss white mice, weighing 35–45 g. Mice were housed in an air-conditioned vivarium, grouped 5 per standard plastic cage with bedding. The light cycle was 12 hours light, 12 hours dark. Mice had been habituated to their room and to human contact for at least one month, and had continuous access to food and water. All testing was conducted during their light cycle, between 0900 and 1300 hours.

Induction of Catalepsy

As in a previous study [13] mice were tested for catalepsy by the common method of placing them head down, approximately midway on a 39-cm tall×25 cm wide sheet of hardware cloth (5 sq cm grid) that was braced at a 45° angle. At the top of the grid, a vertical panel prevented mice from walking over the top of the grid; they could not leave the sides without falling to the floor. Access off the grid was via the bottom, which led to a plastic "home cage" containing the bedding to which they were accustomed. Mice were gently removed from their home cage by their tail and placed midway on the grid.

Under these conditions, normal undrugged mice quickly scurried down to the "home cage" at the bottom of the grid usually within 1–2 sec. Drug-induced catalepsy was scored in terms of the number of seconds mice stayed on the grid and

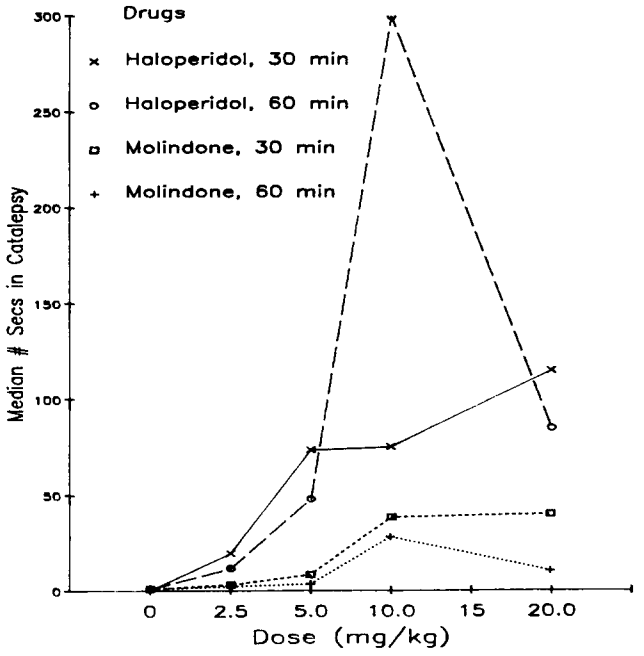

FIG. 1. Cataleptic dose responses of mice to various doses of haloperidol and molindone, with mice tested at 30 and 60 min after a single injection of a given dose of each drug (n=10 for each drug at each dose). Data are expressed in terms of the median time of immobility on the wire grid for each group of mice. Both drugs were clearly cataleptogenic at both 30 and 60 min, with haloperidol being distinctly more potent, especially at 30 min.

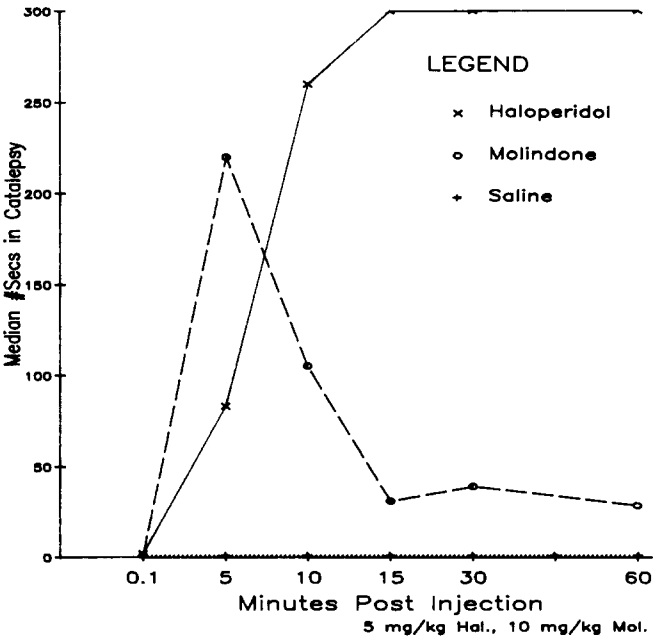

FIG. 2. Comparison of the duration of cataleptogenic effect of the two drugs in mice that were repeatedly tested at successive times after the injection. Repeated testing of saline controls disclosed no signs of catalepsy at any time. Molindone's effect abated after 5–10 min, whereas haloperidol catalepsy became progressively pronounced (more so than when the same dose was given to mice that were not repeatedly tested—see Fig. 1).

in terms of the % of the mice tested which reached a criterion level of staying on for more than 60 sec.

Mice were considered cataleptic rather than simply akinetic because they typically clutched at the wire with their claws and sustained abnormal postures in which the limbs were displaced variously in all directions.

Drugs and Experimental Design

Molindone was prepared fresh daily in sterile saline in a concentration that permitted the same small volume of drug solution (0.1 ml/10 g) to be injected as was injected with the saline control injection and with the commercially available injectable haloperidol (Haldol, McNeill). All injections were intraperitoneal.

Dose-response comparisons were made at 30 and 60 min post injection between molindone and haloperidol, and both drugs were compared for the time course of their effects. In addition, another set of experiments compared the effects of molindone and haloperidol in mice that were given apomorphine at the same time. Each drug was injected via separate syringe and needle; apomorphine solutions had ascorbic acid added (1 mg/ml) to minimize decomposition.

Ten mice were used in each dose or treatment group.

Five repeated tests during 60 min of the same 10 mice after a saline injection produced essentially the same scores of time on the grid (1–3 sec). Thus, there was no indication of a "training" effect, and therefore, the same experimental mice were used at four-day intervals for up to three drug treatments, selected randomly.

Statistical Analysis

Because the drugs caused some mice to remain cataleptic longer than the arbitrary cut-off time of 300 sec, nonparametric statistics were used (SAS software). Data were recorded in terms of the time spent immobile on the grid and the % of mice that remained immobile there for more than 1 min. Comparisons were evaluated by the Kruskal-Wallis test.

RESULTS

Dose-Response Data

Both molindone and haloperidol were cataleptogenic, in terms of the percentage which stayed on for longer than 1 min and in terms of the median length of time mice stayed on the grid (Fig. 1). Saline-injected mice that were tested this same way remained on the grid with median times of 1 sec, both at 30 and 60 min post-injection; no control mice reached the 60-sec criterion of catalepsy.

Both in terms of median catalepsy times and percentage of mice that were cataleptic, the results were highly significant statistically ($p<0.0001$), even for the 60-min molindone data. Moreover, haloperidol was more potent than molindone, in terms of mg/kg, both in terms of median catalepsy time and percentage reaching the cataleptic criterion. The apparently deviant data point after 20 mg/kg of haloperidol is probably misleading; several of these mice had low catalepsy scores because they slid off of the grid, rather than walking off quickly as normal mice do.

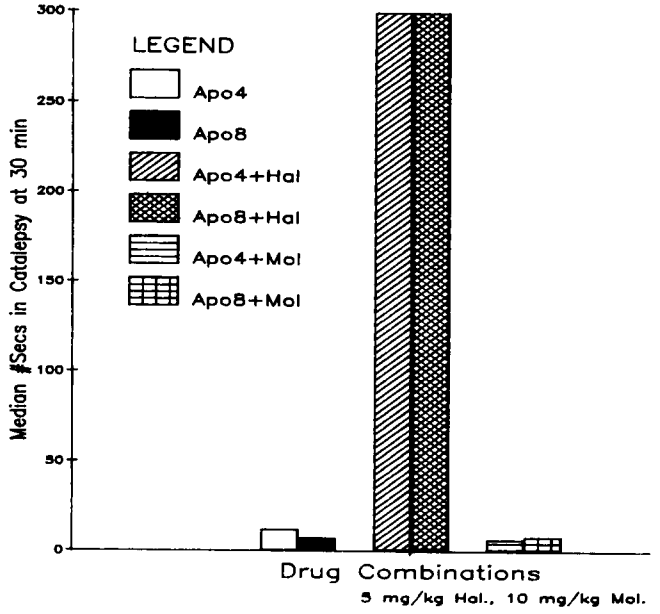

FIG. 3. Potentiation of haloperidol-, but not molindone-induced catalepsy by concurrent injection of apomorphine. Both doses of apomorphine caused a pronounced enhancement of haloperidol-induced catalepsy, but there was no sign of any potentiation of molindone. Control values for when haloperidol and molindone were used alone can be seen in Fig. 1.

Time Course

The duration of molindone's cataleptogenic effect was relatively short, compared to haloperidol, even though the molindone dose used was twice as large. The molindone catalepsy was mostly limited to the first 10 min post injection in terms of median time on the grid (Fig. 2) and to the first 30 post injection min in terms of the percentage of mice that met the 60-sec criterion ($p<0.0001$). Haloperidol's cataleptogenic effect was more prolonged than that of molindone, by both measures. There is a strong indication of a "training effect" with haloperidol, but not saline or molindone, in that haloperidol caused maximum catalepsy (>300 sec median at 30 min post injection) (Fig. 2) when the same mice were tested repeatedly, whereas when mice were tested for the first time at 30 min after the same 5 mg/kg, the median No. sec was less than 50 (Fig. 1). This training effect is reminiscent of a report that rats that were repeatedly tested in separate sessions for haloperidol-induced catalepsy had much higher catalepsy scores than when control rats were tested only once [21].

Interactions with Apomorphine

The 5 mg/kg dose of haloperidol used produced a mild degree of catalepsy when mice were tested only twice at 30 and 60 min (Fig. 1). As expected, when apomorphine was given alone (paired with a saline control injection), there was no obvious cataleptic effect (Fig. 3). But when either dose of apomorphine was given at the same time as haloperidol, there was a marked potentiation of catalepsy, both at 30 and 60 min post injection (30-min data shown in Fig. 3). Notably, this potentiation did not occur when molindone was the simultaneously administered neuroleptic ($p<0.0003$).

DISCUSSION

Molindone was definitely cataleptogenic, due presumably to its preferential blockade of D_2 receptors. It should be noted that previous reports [9,15] have indicated that D_2 blockers such as molindone or sulpiride have little or no cataleptic effect. Whether this reflects a species difference or methodological differences is unclear; sulpiride's weak ability to cause catalepsy appears to be due to poor penetration of the blood-brain barrier [8]. The important point demonstrated by these present data is that a relatively pure D_2 blocker is cataleptogenic.

Molindone was much less cataleptogenic than haloperidol, both in terms of magnitude and duration of effect. Perhaps this resulted because haloperidol apparently has a much greater binding potency for D_2 receptors than do drugs like molindone [7,10].

Conclusions about the dopamine receptor which mediates catalepsy need to be assessed in light of the potentiation of haloperidol catalepsy by concurrent administration of apomorphine. Apomorphine's potentiation of haloperidol-, but not molindone-induced catalepsy, is very difficult to explain in terms of D_1 and D_2 receptors, as they are presently understood. One might have explained the apomorphine potentiation of haloperidol catalepsy on the basis that agonist action on D_1 receptors is cataleptogenic; this large dose of apomorphine could have gained access to D_1 receptors because they were only partially blocked. Although apomorphine's potency for D_1 receptors is only in the micromolar range [1], we were using large doses of apomorphine. But molindone, has even less blocking ability for D_1 receptors than haloperidol, yet apomorphine did not potentiate molindone catalepsy.

Apomorphine is only a partial agonist and has some antagonist properties, particularly for D_1 receptors. For example, at *high* concentrations, which were probably achieved by the large doses that were used here, apomorphine does not stimulate and may even block D_1 receptor-mediated adenylate cyclase responses in striatum [11] and in bovine parathyroid preparations [3]. This implies that blockade of D_1 receptors also contributes to catalepsy. This could also explain why haloperidol is more cataleptogenic than selective D_2 blockers because it blocks both D_1 and D_2 receptors. The role of D_1 blockade should be tested with a selective D_1 blocker.

We have shown that a *low* dose of apomorphine (0.3 mg/kg, IP) can by itself cause a short-lasting post-injection period of catalepsy in mice [13]; a similar finding has also been reported in rats [2]. Bromocriptine, a selective D_2 agonist, is apparently not cataleptogenic, and we have found that it does not potentiate haloperidol catalepsy [13]. Notably, in the rat experiments referenced above, low doses of molindone (0.45 or 0.8 mg/kg, IP) *blocked* rather than enhanced the apomorphine-induced catalepsy, and that parallels our demonstration here that apomorphine does not cause catalepsy in molindone-treated mice. A finding that is probably related is the report that molindone, but not sulpiride, also failed to antagonise the locomotor inhibition that was produced by low doses of apomorphine in mice [12].

Molindone has some characteristics that are not shared by D_1 and D_2 blockers such as haloperidol. For example,

molindone does not increase the firing rate of nigrostriatal DA neurons as does haloperidol [4,5]. Chronic administration of molindone apparently does not promote striatal DA receptor supersensitivity, as does chronic haloperidol [20]. In rats given 2.5 mg/kg, molindone reportedly acts like a typical D_2 blocker (activation of tyrosine hydroxylase, increased DA metabolite levels), but at 40 mg/kg it had opposite effects which lasted up to 72 hr. Intermediate effects were seen at 10 mg/kg; note that 10 mg/kg for a rat is metabolically a larger dose than 10 mg/kg for a mouse. At the higher doses monoamine oxidase was inhibited [17].

Evidence for molindone's low potency for blocking D_1 receptors includes observations, for example, that it is several hundred times less active than most phenothiazines in inhibiting striatal DA-sensitive adenylate cyclase [14]. Molindone's lack of D_1 potency is comparable to that of metoclopramide and sulpiride [9]. Another study showed that molindone in concentrations of 10 mM was totally insensitive in displacing spiroperidol binding (to D_2 receptors), whereas micromolar concentrations of haloperidol were very effective in displacing spiroperidol [22].

In vitro binding studies have their problems, and unfortunately too many of our concepts are based exclusively on such data. We need more functional *in vitro* studies of the kind recently reported which showed that apomorphine had much greater postsynaptic receptor potency (inferred from inhibition of acetylcholine and glutamate release in striatal slices), while bromocriptine had a much greater potency for autoreceptors (depression of DA synthesis) [24].

The present results may have been influenced by the fact that haloperidol (in doses >0.8 mg/kg in rats) has effects on other neurotransmitter systems, such as decreased serotonin in the raphe nuclei; apomorphine increases serotonin fluorescence and 5-HIAA levels in the dorsal raphe and striatum [16]. Molindone also has some other transmitter effects; for example it blocks the cyclic AMP response to norepinephrine in limbic forebrain [18].

Proper interpretation of these present results must await a better understanding of the different kinds of DA receptors in the brain. A complication is that dopaminergic autoreceptors are not well understood in the D_1/D_2 classification scheme [1]. There is still controversy over whether these are a form of D_2 receptor, or another undiscovered class of DA receptor [24]. Recent binding affinity studies have led to the suggestion that autoreceptors are D_2 receptors with especially high affinity for dopamine and apomorphine; D_2 receptors may exist in either a high- or a low-affinity state, which differ on the order of 200-fold in binding affinity for apomorphine [19]. The comparative potency of molindone and haloperidol on these two binding states has not been fully evaluated.

One assay for autoreceptor activity indicated that molindone was more effective in blocking autoreceptors (high-affinity D_2 receptors?) than postsynaptic (low-affinity D_2?) receptors [1]. If autoreceptors are of high-affinity D_2 type, then it would indicate that molindone has low blocking potency on low-affinity receptors. Low blocking potency has also been inferred from studies which tested various neuroleptics for ability to induce supersensitivity to dopamine agonists in mice. Haloperidol, as expected, elevated binding after 21 days of chronic administration; clinically equivalent doses of molindone did not produce supersensitivity [20].

If we assume that catalepsy is mediated by agonist binding at high-affinity D_2 sites (autoreceptors), then several of these present results can be explained. Low doses of apomorphine could preferentially bind high-affinity sites, thus causing catalepsy. Higher doses would load the low-affinity D_2 sites as well, which if they mediated a conflicting behaviour of movement, would obliterate any cataleptogenic effect mediated by the high-affinity D_2 sites. Haloperidol should be more cataleptogenic than molindone, if haloperidol were more potent at low-affinity D_2 sites, leaving some high-affinity D_2 sites free to respond to endogenous dopamine. Molindone, by selectively blocking high-affinity D_2 sites, would thus prevent the enhancement of catalepsy normally caused by apomorphine. Bromocriptine's apparent lack of cataleptogenic effect under any circumstances might indicate that it has a very low capacity for binding high-affinity D_2 receptors.

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