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Behavioural Synergism Between the Dopamine Agonists SKF 38393 and LY 171555 in Dopamine-Depleted Mice: Antagonism by Sulpiride Reveals Only Stimulant Postsynaptic D-2 Receptors

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STARR, M. S. AND B. S. STARR. *Behavioural synergism between the dopamine agonists SKF 38393 and LY 171555 in dopamine-depleted mice: Antagonism by sulpiride reveals only stimulant postsynaptic D-2 receptors.* PHARMACOL BIOCHEM BEHAV **33**(1) 41–44, 1989.—The behavioural effects of the D-1 agonist SKF 38393 (0.56–45 mg/kg) and the D-2 agonist LY 171555 (0.05–6.25 mg/kg) were studied in mice rendered akinetic with a combination of reserpine and α-methyl-p-tyrosine (α-MPT). Under these conditions both agonists were behaviourally ineffective by themselves, but interacted synergistically to restore locomotor and orofacial movements. High levels of D-1 stimulation did not promote stereotypies at the expense of locomotion in dual reserpine- and α-MPT-treated mice, suggesting that the concomitant stimulation of D-1 receptors is essential both for the genesis and subsequent development of all components of D-2 behaviours. The motor stimulant effects of LY 171555 (0.05–1.25 mg/kg), administered in conjunction with a near-maximal dose of SKF 38393 (15 mg/kg), were dose-dependently inhibited by sulpiride at all doses tested (1, 10 and 50 mg/kg). The augmentation of D-2 behaviours by low doses of sulpiride, noted in an earlier study, was not observed here. Results with this particular combination of drugs therefore do not support the concept of mixed excitatory and inhibitory postsynaptic D-2 receptors. The results are discussed in terms of different D-2 agonists interacting with discrete subpopulations of D-2 receptors at postsynaptic sites.

Reserpine α-Methyl-p-tyrosine D-1 receptors D-2 receptors Motor behaviour

RECENT evidence suggests that the suppression of intrinsic dopaminergic activity by dopamine (DA) autoreceptors may not fully account for the sedative properties of DA agonists. For example, it has been shown that DA D-1 receptor agonists promote locomotion in chronically-reserpinised, DA-supersensitive mice, and that this effect can be antagonised by DA D-2 receptor agonists (9,14). Since the reserpine treatment reduced both striatal and limbic DA levels by >96% (14), it is considered unlikely that the D-2 agonists were acting presynaptically to inhibit movement in these experiments.

Rubinstein *et al.* (10) also found that the mixed D-1/D-2 agonist, pergolide (7), restored movement to mice treated acutely with a mixture of reserpine and the DA synthesis inhibitor α-methyl-p-tyrosine (α-MPT), this effect of pergolide being enhanced by low doses, and antagonised by high doses, of the D-2 receptor blocking drug sulpiride (8). These data were presented as evidence for the existence of two distinct types of postsynaptic D-2 receptors with opposing behavioural actions, one type inhibiting locomotion and the other promoting it.

In the present study we have used a similar experimental protocol in an attempt to investigate these purported D-2 receptor subtypes in greater detail. For this purpose, various combinations of the selective D-1 agonist SKF 38393 (11,12) and the selective D-2 agonist LY 171555 (11,15) were used, and their sensitivity to the effects of sulpiride examined under different conditions of D-1 and D-2 stimulation.

METHOD

Behavioural Testing

Male albino mice (A. R. Tuck Ltd.), weighing 35–45 g, were housed in groups of ten in temperature-regulated surroundings, under fluorescent lighting from 09.00–17.00 hr, and allowed free access to food and water. Behavioural testing was carried out between 09.30 and 15.30 hr.

At the outset of the experiment (t = 0 min) mice were placed singly onto the floor of a Perspex container (30 × 35 × 20 cm high) and their locomotor activity measured automatically by under-

TABLE 1

BEHAVIOUR PATTERNS EXHIBITED BY DUAL RESERPINE- AND α-MPT-TREATED MICE FOLLOWING SYSTEMIC INJECTION OF LY 171555 AND SKF 38393, ALONE AND IN COMBINATION

	Frequency of Response				
Behaviour	LY 6.25	SKF 15	LY 0.25 + SKF 15	LY 1.25 + SKF 15	LY 6.25 + SKF 15
Still	10/10	10/10	0/10*	0/10*	0/10*
Rearing	0/10	0/10	8/10*	9/10*	8/10*
Grooming	0/10	0/10	9/10*	10/10*	0/10
Licking	0/10	0/10	2/10	5/10‡	4/10‡
Sniffing	0/10	2/10	9/10*	10/10*	8/10*
Gnawing	0/10	0/10	0/10	0/10	6/10†

LY=LY 171555, SKF=SKF 38393. All drug doses given in mg/kg. *$p<0.005$, †$p<0.01$, ‡$p<0.05$ versus LY or SKF alone by Fisher Exact Probability test (results for LY 171555 0.25 and 1.25 mg/kg alone are not shown, but were indistinguishable from those for 6.25 mg/kg).

floor sensors, over a period of 10 min, using Panlab equipment as described in detail elsewhere (13). Rearing and orofacial movements were also noted as being present or absent. The mice then received α-methyl-p-tyrosine (200 mg/kg at t = 10 min, 100 mg/kg at t = 190 min) and reserpine (5 mg/kg at t = 70 min), as well as various doses of water (controls, at t = 220 min), sulpiride (at t = 205 min), SKF 38393 (at t = 220 min) or LY 171555 (at t = 230 min) as indicated in the text. All injections were delivered in a volume of 5 ml/kg intraperitoneally, except for LY 171555 which was injected subcutaneously in the neck region. The mice were retested at t = 190, 250 and 310 min.

Drug effects were compared by two-way analysis of variance and individual drug doses were also subjected to post hoc analysis using Duncan's test. Rearing and stereotypy scores were compared by Fisher Exact Probability test.

Drugs

Reserpine (Sigma) and sulpiride (donated by Chemitechna) were dissolved in one drop of glacial acetic acid and subsequently diluted with demineralised water. α-Methyl-p-tyrosine (Sigma), SKF 38393 (Research Biochemicals Inc.) and LY 171555 (donated by Lilly) were dissolved in water alone.

RESULTS

On the first testing, prior to reserpinisation, mice averaged 863.1 ± 8.2 locomotor counts (mean ± SEM, n = 60) over the 10-min observation period. These perambulatory movements were interspersed initially by exploratory rearing (10/10 mice) and sniffing (10/10), and later by extended bouts of grooming (10/10) and stillness [10/10, see (13)]. Licking (0/10) and gnawing (0/10) did not occur spontaneously. Two hours after reserpine all animals appeared heavily sedated (mean 44.4 ± 5.2 counts) and at 3 hr were completely immobile (mean 0.6 ± 0.1 counts for water-injected controls). All other species-typical motor behaviours likewise ceased after reserpine.

Neither the D-1 agonist SKF 38393 (0.56–45 mg/kg), nor the D-2 agonist LY 171555 (0.05–6.25 mg/kg), administered singly to reserpine- plus α-MPT-treated mice, were able to reinstate motility to any significant extent (Figs. 1 and 2, Table 1).

By contrast, mixtures of the two agonists actively restored locomotion and orofacial movements, the level and the character

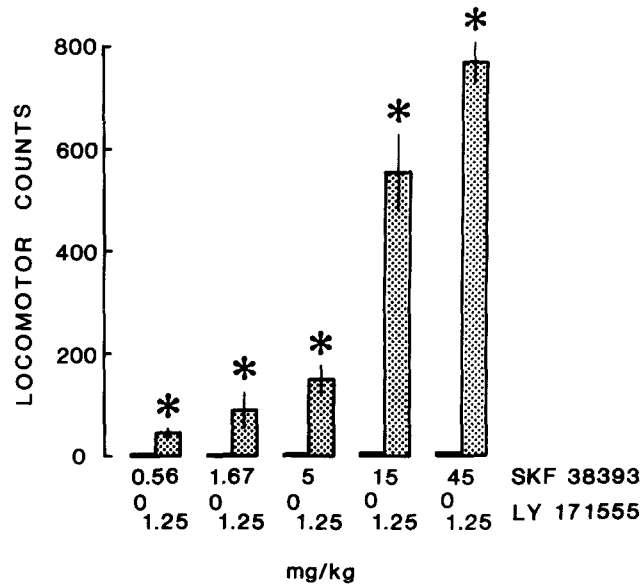

FIG. 1. Locomotor activity induced by varying doses of SKF 38393 administered together with a fixed dose of LY 171555 in dual reserpine- and α-MPT-treated mice. Results are means ± SEM (n = 10) for SKF 38393 injected alone (filled columns) and in the presence of 1.25 mg/kg LY 171555 (stippled columns). *$p<0.001$ versus SKF 38393 alone by Duncan's test.

of the response both depending on the D-1/D-2 dose ratio. Figure 1 shows that increasing amounts of SKF 38393 (0.56–45 mg/kg), injected together with an intermediate dose of LY 171555 (1.25 mg/kg), dose-dependently increased the frequency of the animals'

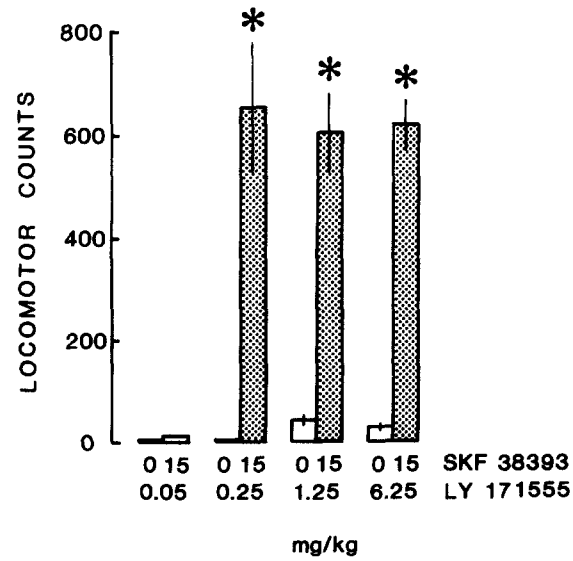

FIG. 2. Locomotor activity induced by varying doses of LY 171555 administered together with a fixed dose of SKF 38393 in dual reserpine- and α-MPT-treated mice. Results are means ± SEM (n = 10) for LY 171555 injected alone (open columns) and in the presence of 15 mg/kg SKF 38393 (stippled columns). *$p<0.001$ versus LY 171555 alone by Duncan's test.

locomotor counts, which by observation could be seen to be due to forward walking. These mice showed sustained locomotor activity throughout the whole of the observation period, with none of the periods of stillness typical of within-session habituation that we saw prior to treatment with reserpine and α-MPT. As the D-1 agonist dose was raised to 15 mg/kg, ataxia was replaced by a progressive increase in fluidity of the animals' movements, which now closely resembled the locomotion of DA-intact mice. At 45 mg/kg SKF 38393, however, this fluidity gave way to amphetamine-like darting, suggesting overstimulation of the D-1 receptors had shifted the D-1/D-2 balance beyond normal values. The higher doses of SKF 38393 (15 and 45 mg/kg) also gave rise to prominent rearing, sniffing, licking and grooming (Table 1).

When the dose of SKF 38393 was fixed at 15 mg/kg and that of LY 171555 varied (0.05–6.25 mg/kg), a different behavioural profile emerged (Fig. 2 and Table 1). The smallest injection of LY 171555 (0.05 mg/kg) was behaviourally ineffective, whereas a wide spectrum of DA behaviours appeared abruptly as the dose was increased 5-fold to 0.25 mg/kg. These included forward walking with head-down sniffing, rearing and grooming (Table 1). Similar results were obtained with 1.25 and 6.25 mg/kg LY 171555 (Table 1), except that grooming was replaced by gnawing at the highest D-2 agonist dose.

The latter experiment was repeated in the presence of varying amounts of the selective D-2 receptor antagonist, sulpiride (1–50 mg/kg), in an attempt to disclose the hidden motor-inhibiting effect of LY 171555 predicted by the experiments of Rubinstein *et al.* (10). It will be seen from Fig. 3 that in no instance did the blocking drug increase the level of motor responding, in spite of employing a range of D-2 agonist and antagonist doses. On the contrary, only an inhibition of existing locomotion and stereotypy was observed, this being more pronounced at the lower LY 171555 concentrations, and at the later time points, when the effects of the D-2 agonist were wearing off (Fig. 3). Rearing, sniffing and grooming were all reduced in parallel with locomotion; for example, in the presence of 50 mg/kg sulpiride, none of these behavioural elements was observed in mice injected with a combination of 0.25 mg/kg LY 171555 and 15 mg/kg SKF 38393.

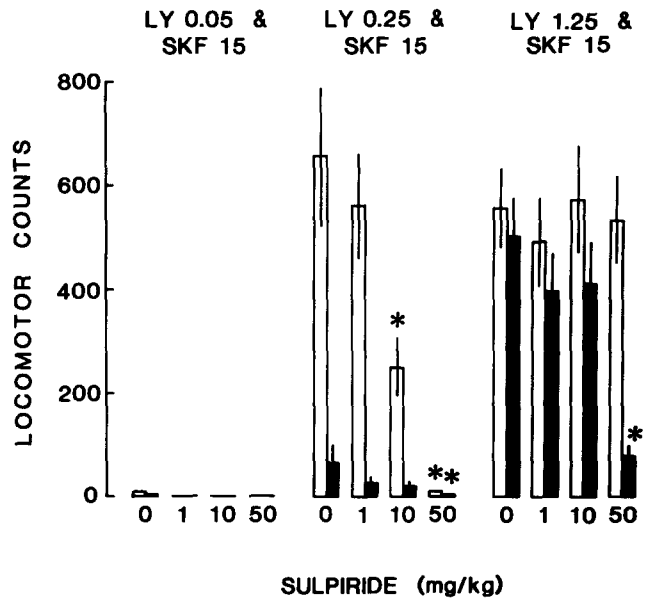

FIG. 3. Effects of sulpiride on the locomotor activity induced by various combinations of LY 171555 and SKF 38393 in dual reserpine- and α-MPT-treated mice. Results are means ± SEM (n=10) of locomotor scores recorded at 30 min (open columns) and 90 min (closed columns) after injecting SKF 38393 (i.e., 20 and 80 min after LY 171555, 45 and 105 min after sulpiride, 180 and 240 min after reserpine respectively). *$p<0.001$ versus corresponding sulpiride-free mice by Duncan's test.

DISCUSSION

From an inspection of the literature, we deduced that treating mice acutely with a combination of reserpine and α-MPT would produce a rodent model that was suitable for specifically investigating the behavioural pharmacology of normotensive, postsynaptic DA receptors, in the absence of any complicating influences of endogenous DA and DA autoreceptors. Under these conditions of DA depletion, we showed that neither the selective D-1 agonist SKF 38393 (11,12), nor the selective D-2 agonist LY 171555 (11,15) was able to evoke any form of behavioural response, whereas a full spectrum of locomotor and orofacial movements developed when the two drugs were administered concurrently. These findings therefore support the view that the generation, intensity and character of D-2 receptor-mediated motor events are all critically dependent on the simultaneous activation of the D-1 receptors (2–6, 9, 14).

Special emphasis has recently been laid on the requirement for intense D-1 stimulation in order to observe stereotyped behaviours with a selective D-2 agonist (1,3). In this situation, locomotion generally becomes arrested, probably because the animals are preoccupied with the execution of stereotyped acts instead. Interestingly, the use of reserpine and α-MPT appeared to preclude the development of intense stereotypies, for although we observed a greater preponderance of sniffing, licking and grooming with the highest dose levels of SKF 38393 (15–45 mg/kg), these movements never become so intense as to prevent the animals rearing and walking about the test arena. On the contrary, the latter steadily increased in frequency over the entire dose range of SKF 38393. This experiment emphasises, therefore, that under certain circumstances high levels of D-1 stimulation can facilitate the expression of all components of DA-dependent behaviours, not just stereotypy.

In addition to the more familiar excitatory postsynaptic D-2 receptor, it is beginning to look as though a second type of D-2 receptor may exist postsynaptically, which is more sensitive to D-2 drugs and is behaviourally inhibitory (9, 10, 14). This would explain the paradoxical hypomotility that is sometimes seen with D-2 agonists (9,14) and the hypermotility produced by D-2 antagonists (10) in DA-depleted animals (i.e., in the absence of autoreceptor effects). Under these conditions, Rubinstein *et al.* (10) showed that akinesia could be reversed by pergolide, and that this effect was potentiated by low doses of sulpiride, presumably because the D-2 antagonist preferentially blocked these inhibitory D-2 receptors. Our attempts to further characterise these hypothetical receptors and their possible D-1 receptor dependency, under closely similar experimental conditions, were less equivocal.

Instead of injecting pergolide, which has a fixed ratio of D-1/D-2 activity (7, 10, 11), we promoted locomotion and orofacial behaviours with mixtures of SKF 38393 and LY 171555, which allowed us to vary the levels of D-1 and D-2 stimulation independently of each other. In this way we found that in the presence of a fixed dose of 1.25 mg/kg LY 171555, SKF 38393 gradually increased motor responding over the dose range 0.56–45 mg/kg, in keeping with the D-1 receptor having a D-2 enabling role (1–3, 5, 6, 14). By contrast, when the concentration of SKF 39393 was held constant at 15 mg/kg, a relatively small increase in the dose of LY 171555, from 0.05 to 0.25 mg/kg, turned a nonresponding mouse into one which exhibited maximum loco-

motion and a wide selection of orofacial movements. In light of previous work (9,10), this rapid switch from complete inertness to total responding could signify that below 0.25 mg/kg, LY 171555 preferentially activates postsynaptic inhibitory receptors (whose effects are not immediately apparent in this model), whilst above this dose level the D-2 agonist also engages postsynaptic excitatory receptors and excitation becomes the predominant response. If this were the case, we reasoned it should be possible to block the inhibitory D-2 component with low doses of sulpiride [as in (10)] and to potentiate these excitatory responses, and possibly even to expose an excitatory phase with the lowest D-2 agonist injection (0.05 mg/kg LY 171555). In this event, sulpiride appeared only to suppress the excitatory responses induced by such mixtures, and never disclosed a hidden inhibitory phase, such as occurred when pergolide was employed as the agonist (10). The reason for this discrepancy is not clear, but one possibility is that pergolide interacts with a family of D-2 receptors that are not accessible to LY 171555, even though the high D-2 selectivity of LY 17155 (11,15) has made it the D-2 agonist of choice in many other behavioural studies (1–3, 5, 6, 9, 11).

It remains to be seen, therefore, whether postsynaptic D-2 receptors can be differentiated pharmacologically, in the same way that D-2 receptors at pre- and postsynaptic sites have been differentiated in the past (11).

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