Pharmacology Biochemistry & Behavior, Vol. 35, pp. 285–289. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Differential Behavioural Interactions Between the Dopamine D-1 Antagonist SCH 23390 and the Dopamine D-2 Antagonists Metoclopramide and Sulpiride in Nonhabituated Mice

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Received 5 September 1989

CHANDLER, C. J., B. S. STARR AND M. S. STARR. *Differential behavioural interactions between the dopamine D-1 antagonist SCH 23390 and the dopamine D-2 antagonists metoclopramide and sulpiride in nonhabituated mice.* PHARMACOL BIOCHEM BEHAV **35**(2) 285–289, 1990.—This study investigated the effects of the selective D-1 antagonist SCH 23390, when administered alone and in combination with a typical (metoclopramide) or atypical neuroleptic (sulpiride), on species-typical behaviours in nonhabituated mice. When tested singly, all three compounds caused a progressive dose-dependent inhibition of locomotion, rearing and grooming, though their potencies varied widely. Mixtures of a threshold dose of 0.01 mg/kg SCH 23390 and metoclopramide (0.05–1.25 mg/kg) interacted synergistically to promote hypomotility and to decrease rearing, but did not affect grooming. By contrast, combinations of 0.01 mg/kg SCH 23390 and sulpiride (2–10 mg/kg) resulted in a marked potentiation of grooming, but only additive reductions in horizontal and vertical movements, consistent with sulpiride and metoclopramide occluding different populations of D-2 receptors. The results show that blockade of D-1 receptors and certain populations of D-2 receptors can interact positively to modify animal motor behaviour, and add a fresh perspective to the concept that these two types of dopamine receptor normally work interdependently to control movements of the body.

Mice Motor behaviour Dopamine D-1 receptors D-2 receptors SCH 23390 Metoclopramide Sulpiride

THE subclassification of brain dopamine receptors into D-1 and D-2 subtypes is based on biochemical criteria (19). And yet, paradoxically, the performance of selective agonists and antagonists of these receptors in behavioural models has revealed aspects of their functional organisation which could not have been anticipated from a knowledge of their biochemistry [for reviews see (3, 20, 21)]. A major revelation of motor studies has been the discovery that D-1 receptors play a crucial accessory role in the regulation of motility by D-2 receptors, leading to a reevaluation of antiparkinson treatment in man (3,21).

While the facilitation of D-2 responses by D-1 agonists is a widely accepted phenomenon, the nature of this functional D-1/D-2 coupling remains unclear. Current evidence argues against it occurring within the same cell (7), even though it appears that D-1 and D-2 receptors can coexist in the same membrane (23). This has led several investigators to propose that D-1/D-2 interactions observed in behavioral (3, 20, 21) and electrophysiological experiments (7,22) involve D-1 and D-2 receptors on separate neuronal systems, which then interact via convergent output pathways (6, 7, 13).

A similar conclusion has been reached in studies with selective D-1 and D-2 antagonists, which are believed to act at different sites to suppress motor acts (11,12), but whose abilities to influence each other's actions in combination experiments are virtually unknown. A recent report by Klemm and Block (9) concluded that SCH 23390 (a D-1 blocker) (8) worked additively with molindone (a D-2 blocker) to produce hypomotility in rats, which is contrary to biochemical predictions (15). A more detailed investigation of how selective D-1 and D-2 antagonists interact in vivo, to modify the animal's behaviour and brain biochemistry,

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could, therefore, yield new information concerning the site(s) and mechanism(s) of the phenomenon of D-1/D-2 receptor cooperativity in the brain.

In this first report we examine the motor responses of nonhabituated mice treated with SCH 23390 to block brain D-1 receptors, both alone and in conjunction with a typical (metoclopramide) or atypical (sulpiride) D-2 receptor-blocking neuroleptic. The results show that both neuroleptics interact positively with SCH 23390, but in different ways.

METHOD

Animals and Testing Procedure

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 conducted between 10.00 and 15.30 hr.

Animals were injected with water (vehicle controls, at t = 30 min), SCH 23390 (at t = 30 min), sulpiride (at t = 0 min) or metoclopramide (at t = 30 min), alone or in combination, then returned to their home cage. At t = 60 min, mice were placed singly onto the floor of a clear Perspex box (30 × 35 × 25 cm high) and their horizontal movements measured automatically by underfloor sensors, over a period of 10 min, using Panlab equipment as described in detail elsewhere (17). Rearing frequency was scored by direct observation with hand-held counters, and consisted of the animal lifting both forepaws off the ground, either supported against a side wall, or unsupported in mid-air. Grooming time (sec) was evaluated either directly or from playbacks of video recordings.

Drug effects were compared by one-way or two-way analysis of variance (ANOVA) of raw data, to determine F-ratios and p-values, followed by post hoc analyses by Duncan's or Dunnett's tests. Results are expressed graphically as actual scores for SCH 23390 administered alone, and as % appropriate controls for metoclopramide and sulpiride given alone (control = water) and with SCH 23390 (control = 0.01 mg/kg SCH 23390).

Drugs

Gifts of sulpiride (Chemitechna), metoclopramide (Beecham) and SCH 23390 (Schering) are gratefully acknowledged. All drugs were dissolved in demineralised water and injected intraperitoneally in a dose volume of 5 ml/kg. The solution of sulpiride was aided with one drop of glacial acetic acid before dilution in water.

RESULTS

Nonhabituated control mice, injected with demineralised water, averaged 953.4 ± 15.9 locomotor counts, 74.8 ± 7.3 rears and 86.9 ± 8.9 sec grooming during the course of the 10-min observation period (Fig. 1).

The behavioural profile of the D-1 antagonist SCH 23390, administered singly over the dose range 0.01–1.25 mg/kg, is shown in Fig. 1. As the amount of the benzazepine was increased, all of the recorded behaviours were steadily depressed. From these data, an injection of 0.01 mg/kg SCH 23390 was identified as being a threshold dose, suitable for further study in combination with D-2 antagonists (see below).

Behavioural profiles were similarly obtained for the D-2 antagonists metoclopramide (0.05–6.25 mg/kg) and sulpiride (2–50 mg/kg), as indicated in Figs. 2 and 3. Metoclopramide caused a significant ($p<0.05$), though invariant reduction in horizontal (8–11% inhibition) and vertical movements (20–23% inhibition)

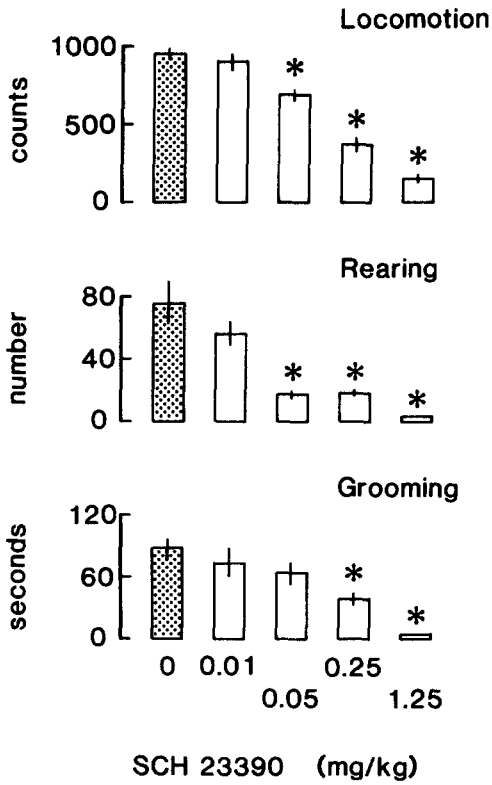

FIG. 1. Dose-related changes in mouse locomotor activity, rearing and grooming elicited by the dopamine D-1 antagonist SCH 23390. Stippled columns refer to vehicle controls. The threshold dose of 0.01 mg/kg SCH 23390 was selected for subsequent drug combination studies and represents the control for the stippled columns depicted in Figs. 2 and 3. Results are means ± SEM of 8–9 experiments. *$p<0.05$ versus vehicle controls by Dunnett's test.

over the dose range 0.05–1.25 mg/kg, followed by a much more profound abolition of these activities at 6.25 mg/kg (70–75% inhibition, Fig. 2). Metoclopramide did not affect grooming at the lowest doses, but the highest dose inhibited it (56% inhibition, $p<0.05$, Fig. 2).

The effects of sulpiride were clearly different from those of metoclopramide (Fig. 3). With sulpiride, grooming was preferentially depressed at 2 mg/kg (35% inhibition, $p<0.05$), this being accompanied by reductions in locomotion (16–69%, $p<0.05$) and rearing (37–97%, $p<0.05$) at 10 and 50 mg/kg sulpiride respectively.

To determine the effects of combined D-1 and D-2 receptor blockade, further groups of mice were injected with a fixed dose of SCH 23390 (0.01 mg/kg) together with one of a range of doses of either D-2 antagonist.

In the presence of 0.01 mg/kg SCH 23390, metoclopramide now induced a dose-dependent decrease in locomotor and rearing scores over the entire dose range 0.05–1.25 mg/kg (Fig. 2). Within-treatment differences for metoclopramide were not significant ($p>0.05$) for locomotion, $F(3,36) = 1.76$, or rearing, $F(3,36) = 1.67$, when the drug was administered on its own, but both became highly significant ($p<0.01$) when the D-2 antagonist was coinjected with SCH 23390 [$F(3,36) = 14.54$ for locomotion, $F(3.36) = 9.28$ for rearing]. These results are consistent with the two treatments interacting synergistically to modify the animals' horizontal and vertical movements.

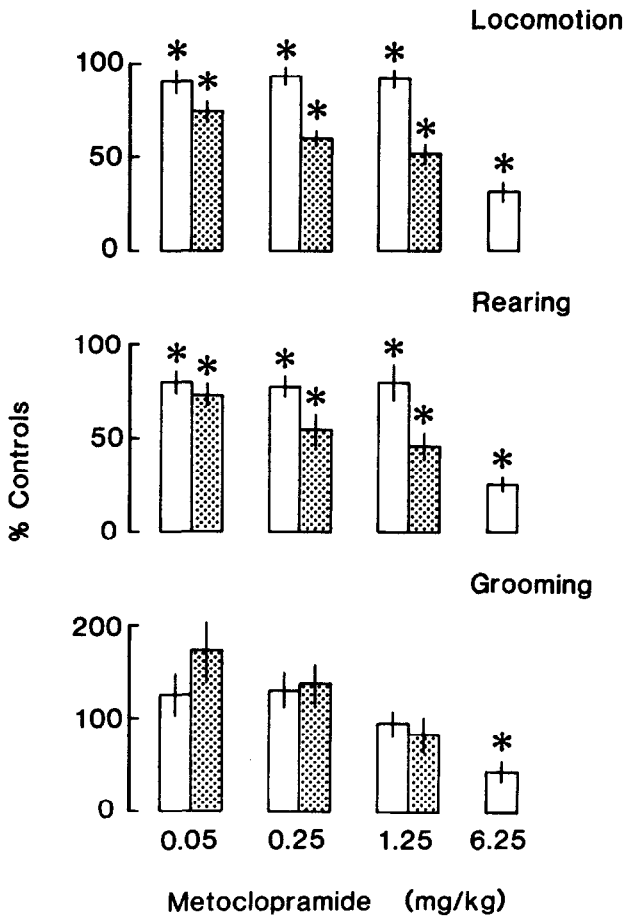

FIG. 2. Dose-related effects of the D-2 antagonist metoclopramide on motor behaviour in the nonhabituated mouse, in the absence (open columns, controls = water) and presence of 0.01 mg/kg SCH 23390 (stippled columns, controls = 0.01 mg/kg SCH 23390). Results are the means ± SEM of 8–10 experiments. *$p<0.05$ versus appropriate controls by Dunnett's test.

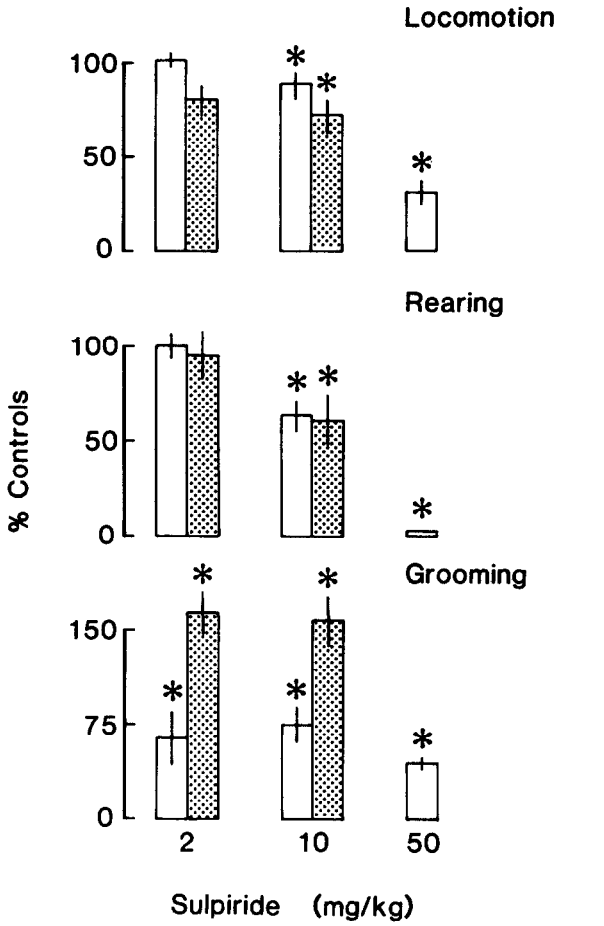

FIG. 3. Dose-related effects of the D-2 antagonist sulpiride on motor behaviour in the nonhabituated mouse, in the absence (open columns, controls = water) and presence of 0.01 mg/kg SCH 23390 (stippled columns, controls = 0.01 mg/kg SCH 23390). Results are means ± SEM of 14 experiments. *$p<0.05$ versus appropriate controls by Dunnett's test.

By contrast, we found no evidence of the two drugs interacting to affect grooming (Fig. 2). Two-factor ANOVA showed metoclopramide, 0.05–1.25 mg/kg, had no significant effect on the time the mice spent grooming, either when given alone, $F(3,36) = 1.61$, $p = 0.20$, or with 0.01 mg/kg SCH 23390, $F(3,36) = 0.01$, $p = 0.91$.

Figure 3 illustrates a different type of interaction occurred between SCH 23390 and sulpiride. The interaction terms calculated by two-factor ANOVA revealed that the effects of sulpiride, 2–10 mg/kg, on locomotion, $F(2,73) = 0.68$, $p = 0.51$, and rearing, $F(2,73) = 0.72$, $p = 0.49$, were not significantly altered by the presence of 0.01 mg/kg SCH 23390. On the other hand, and in contrast to metoclopramide, SCH 23390 plus sulpiride caused a pronounced increase in grooming time compared to either drug alone ($p<0.05$), which could not be accounted for by a simple addition of individual effects [$F(2,73) = 3.64$ for the drug-drug interaction, $p = 0.03$].

DISCUSSION

The present data indicate that SCH 23390, a selective antagonist of dopamine D-1 receptors (8), interacts differentially with the D-2 antagonists metoclopramide and sulpiride to modify the motor behaviour of nonhabituated mice. The D-2 blockers, both substituted benzamides, were chosen for their different behavioural spectra. Metoclopramide is classified as a typical neuroleptic (though whether it is antipsychotic is questionable), while sulpiride is an atypical neuroleptic (4, 10, 16), emphasising the two compounds act preferentially at different D-2 sites within the brain. SCH 23390, on the other hand, resembles typical neuroleptics in behavioural models (2, 3, 20), yet has the in vivo biochemical profile of an atypical neuroleptic (1), making it difficult to anticipate what form any interaction between SCH 23390 and these two D-2 blocking drugs would take.

What we found in practice was that SCH 23390 administered in conjunction with metoclopramide, but not sulpiride, inhibited the animals' horizontal and vertical movements (though not grooming) to a much greater extent than we would have predicted from a simple summation of the drugs' individual responses. These positive interactions occurred with threshold doses of both antagonists, which would, therefore, be expected to preserve their selectivity for the corresponding dopamine receptor subtype in vivo. It is reasonable to conclude, therefore, that the particular population of D-2 receptors which metoclopramide blocks to suppress locomotion and rearing, are in some way cooperatively

linked to D-1 receptors, while those associated with metoclopramide's weaker effects on grooming and the motor responses to sulpiride, are not D-1-dependent.

The mechanism(s) of such D-1/D-2 interactions have been the subject of much speculation. SCH 23390 is known to be a potent inhibitor of motility by itself (2, 3, 20). However, this action is thought to be indirect and due to the blockade of endogenous dopamine released onto D-1 receptors, which are functionally coupled to and normally "enable" the motor stimulant effects of a subpopulation of postsynaptic D-2 receptors (3,19). SCH 23390 can, therefore, be thought of as "disenabling" this cooperative D-1 function. One may consequently hypothesise that the mechanism by which SCH 23390 potentiates hypomotility and depresses rearing in metoclopramide-treated mice probably involves the same neuronal circuitry as that which supports the opposite and much more widely documented phenomenon, namely the facilitation of D-2 agonist-induced increases in motor activity by D-1 agonists (3,20).

In view of the particularly dense association of D-1 receptors with striatonigral neurones (5), this major striatal output pathway has attracted attention recently as a possible route taken by motor information generated at D-1 receptors (6, 11, 13). The striatopallidal projection has similarly been proposed as being proximal to motor-relevant D-2 receptors (6,11). Thus, it is feasible that the D-1/D-2 behavioural interactions observed in this and countless other D-1 and D-2 drug combination experiments [reviewed in (3,20)], reflect the convergence and subsequent integration of separately transmitted striatonigral (D-1-dependent) and striatopallidal (D-2-dependent) motor information at a distant site in the brain, for example the thalamus or the motor cortex (11). Though still highly speculative, this hypothesis has the attraction of avoiding the apparent anomaly that D-1 and D-2 receptors can mediate similar changes in the behaviour of the whole animal, yet can mediate opposite biochemical changes at the cellular level [for fuller discussion see (13)].

The motor inhibitory effects of sulpiride were much weaker than those of metoclopramide and additive with those of SCH 23390, reflecting not only the poor penetration of sulpiride into the brain, but also its preference for a different set of D-2 receptors from those occluded by metoclopramide, which is in accordance with earlier findings (4,12). On the other hand, administering sulpiride and SCH 23390 together resulted in a significantly greater amount of grooming, even though both drugs by themselves inhibited grooming at the doses used. In an analogous experiment, Robertson and MacDonald (12) have previously reported that amphetamine caused a diminution in grooming which could be further depressed by metoclopramide and opposed by sulpiride. Presumably, by blocking D-2 receptors that normally oppose this behaviour, sulpiride is unmasking a D-1 receptor-mediated amphetamine response. We have noticed in an earlier study that microgram doses of SCH 23390 can apparently promote perseverative grooming in naive mice, in excess of that caused by handling and novelty of the surroundings (18), so perhaps sulpiride was similarly uncovering a latent tendency of the D-1 antagonist to stimulate grooming in the present case. Whatever the mechanism, it would not appear that grooming was heightened by loss of competition from other behaviours, such as walking about the box and rearing, since exaggerated grooming occurred with minimal disruption of these other activities.

The enigma that emerges from this and earlier behavioral studies, in which combinations of dopamine D-1 and D-2 agonists or antagonists have been used, is the singular lack of correlation of the behavioural responses with available in vivo biochemical data. For instance, whereas D-1 and D-2 agonists can be shown to work in concert to increase an animal's movements (3, 19, 21), Saller and Salama (14) have found that the same drugs oppose each other's effects on striatal dopamine turnover. Similar discrepancies come to light when mixtures of D-1 and D-2 antagonists are used. For example, how is it that SCH 23390 and haloperidol (D-2>D-1 blocker) are individually both able to reduce spontaneous motor activity and to elevate dopamine utilisation in the striatum in a dose-dependent fashion, yet when the two drugs are injected simultaneously the behavioural inhibitory actions of SCH 23390 summate with those of haloperidol (Starr and Starr, unpublished data), whereas SCH 23390 paradoxically cancels out the elevating effect of haloperidol on striatal dopamine turnover (15)? Does this mean that striatal dopamine activity is causally related to the behavioural actions of dopaminergic drugs when they are administered singly, but casually related to the behavioural effects the drugs have when they are given in combination? Further comparative behavioural and biochemical studies with D-1/D-2 drug mixtures are required to clarify this point and to disclose the biochemical and anatomical substrate(s) of the D-1/D-2 receptor interactions observed at the behavioural level.

In summary, this study provides fresh evidence for the notion that D-1 and D-2 receptors operate interdependently to regulate motor behaviour, by showing that synergistic drug interactions in a behavioural model are not confined to D-1 and D-2 agonists, but can also be revealed with appropriate antagonists of these receptors. The immediate question that needs to be answered is what is the biochemical basis for this functional effect?

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