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In Morphinised Rats SKF 38393 Converts Dopamine D_2 Receptor-Mediated Forward Locomotion Into Backward Walking

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AXON, D. I. R., G. H. FLETCHER AND M. S. STARR. *In morphinised rats SKF 38393 converts dopamine D_2 receptor-mediated forward locomotion into backward walking.* PHARMACOL BIOCHEM BEHAV **26**(1) 45–47, 1987.—The behavioural responses to RU 24213 (D_2 agonist) and SKF 38393 (D_1 agonist) were studied in the morphine cataleptic rat. D_2 stimulation evoked slow forward walking coupled with head-down sniffing that was blocked both by SCH 23390 (D_1 antagonist) and metoclopramide (D_2 antagonist). D_1 stimulation was without effect by itself, but when administered together with RU 24213 it reversed the direction of walking and initiated licking and chewing. Backward walking was attenuated by metoclopramide and restored to forward locomotion by SCH 23390. These data further show that D_1 receptors exert an important modulatory influence on motor behaviours mediated by the D_2 site.

Morphine Catalepsy SKF 38393 RU 24213 Backward walking

MORPHINE has complex and species-variable effects on motor behavior, which are often in opposition to those elicited by dopaminomimetics [11]. In the rat, morphine alters motility biphasically [9,14], though in high doses it reliably produces a state of rigid catatonia that can be overcome by administering apomorphine [6]. Whether this effect is mediated by the D_1 or D_2 subclass of dopamine receptor, or both, is unclear as apomorphine is capable of stimulating both types [12,17]. Given the recent hypothesis that dopamine behaviours are principally D_2-mediated events that require ongoing D_1 stimulation for their full expression, both in normal animals [7, 10, 15] and following depletion of dopamine by reserpine [1, 4, 16], we considered it of interest to investigate how selective D_1 and D_2 agonists administered separately and in combination, modify the state of morphine catalepsy in the rat.

In this report we describe an unusual backward locomotor response that appears when the D_1 agonist SKF 38393 [13] is injected in conjunction with the D_2 agonist RU 24213 [3] in morphinised rats. These results offer further evidence of a functional interaction between D_1 and D_2 receptors.

METHOD

Behavioural Testing

Female Wistar albino rats (Olac Ltd.) weighing 140–160 g were injected subcutaneously (SC) with 30 mg/kg morphine sulphate (MacFarlan Robinson). This was found to be the minimum dose which rendered all rats cataleptic after 45 min, as determined by their ability to maintain imposed positions for 30 sec in the following tests: (a) Horizontal bar test; the animal's front paws were placed on a steel bar (0.5 cm diameter) elevated 7 cm above the ground; (b) Imposed posture test; the animal was drooped over the same bar with all four paws off the ground; (c) Vertical grid test; the rat was observed for its ability to cling to a vertical wire mesh. Rats scoring positively in each of the three tests were then placed singly onto the floor of a circular test arena (0.84 m diameter, 0.34 m high), the floor of which was marked out in equal areas of 260 cm^2. The capacity of SKF 38393 (Smith, Kline and French) and RU 24213 (Roussel) to reinstate locomotion was determined by injecting the compound SC and 30 min later counting the number of segments entered by the rat over a period of 5 min (i.e., 75–80 min after morphine).

Changes in the direction of movement and in posture, were noted by direct observation. The incidence of orofacial movements was similarly determined and each element of behaviour rated separately according to the following scale: 0=absent, 1=weak and sporadic, 2=moderate and intermittent, 3=strong and persistent.

In other experiments RU 24213 treatment was given 75 min after morphine and preceded 30 min beforehand by an injection of SKF 38393, SCH 23390 (Schering) or metoclopramide (Beecham). Testing was then conducted at 105–110 min after morphine. In all cases control rats received morphine followed by drug vehicle (distilled water).

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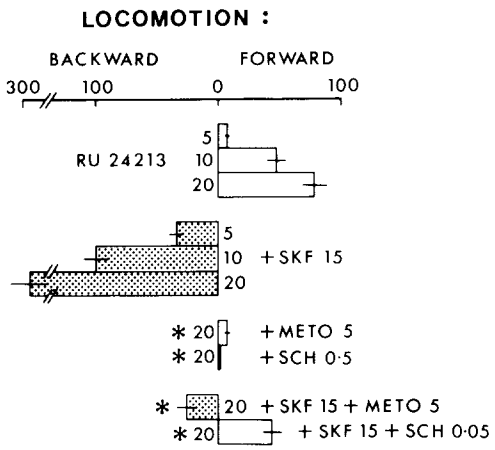

FIG. 1. Effects of D_1 and D_2 agonists and antagonists on locomotor activity of morphinised rats. Results are shown for mean±SEM of 6–8 experiments. Locomotion is expressed as the number of floor segments crossed by the animal in a 5 min observation period. All animals received the D_2 agonist RU 24213 in the doses (mg/kg) shown by the figures. In addition, other groups of rats were pretreated with the D_1 agonist SKF 38393 (+SKF), the D_1 antagonist SCH 23390 (+SCH) or the D_2 antagonist metoclopramide (+METO) in the doses shown (mg/kg). Asterisk indicates $p<0.01$ versus equivalent treatment in the absence of antagonist. SKF 38393 had no effect on locomotion of morphinised rats by itself (not shown).

Statistics

Results were analysed by ANOVA followed by Dunnett's or Duncan's tests.

RESULTS

At 30 mg/kg morphine completely immobilised rats for >3 hr. All locomotion, rearing and other species-typical behaviours, such as sniffing and grooming, were abolished.

The D_1 agonist SKF 38393 failed to modify morphine-induced inactivity, even when doses as high as 15 mg/kg were administered.

By contrast, the D_2 stimulant drug RU 24213 dose-dependently increased locomotion, which consisted of persistent, slow forward ambulation, coupled with intense sniffing of the floor (rating 3/3, Fig. 1). Rearing and grooming were not restored by RU 24213. When rats were treated first with 15 mg/kg SKF 38393, 30 min prior to receiving RU 24213, the D_2 agonist now induced dose-related and continuous backward locomotion, which proved to be more rapid than the forward-directed activity (Fig. 1). The animals continued to assume a hunched posture with their heads pointing downwards and backwards towards their abdomen, resulting in a compulsive and well-coordinated backward shuffling along a direct path until they met the walls of the container, after which the backward locomotion was directed almost exclusively around the perimeter of the circular test box. The net activity resembled a backward version of the ponderous head-down walking induced by RU 24213 in non-morphinised subjects, rather than a reversal of "normal" locomotion *per se*, except the customary sniffing elicited by RU 24213 gave way to exaggerated licking (rating 3/3) and vacuous chewing movements (rating 2/3) in the presence of the D_1 agonist. At no time did the rats make any attempt to switch to forward motion, and they failed to approach or inspect foreign objects (e.g., cork, pencil) placed immediately in front of them.

In order to confirm the involvement of dopamine D_1 and D_2 receptors in the above responses, we repeated the experiments in the presence of SCH 23390 (D_1 blocker) or metoclopramide (D_2 blocker). Small doses of either antagonist attenuated D_2 responding, while higher amounts blocked it completely (Fig. 1); all forward movement was prevented and sniffing was inhibited. Similarly, the backward shuffling and orofacial behaviours evoked by RU 24213 together with SKF 38393 were antagonised by 5 mg/kg metoclopramide. A low dose of SCH 23390 (0.01 mg/kg) slowed backward walking, which was now interspersed with bouts of forward motion. Raising the dose of SCH 23390 (0.05 mg/kg) reversed the direction of locomotion completely and reinstated sniffing in place of licking or chewing (Fig. 1), while a higher dose of SCH 23390 (0.25 mg/kg) blocked all activity (not shown).

DISCUSSION

Several reports have shown recently that while stimulating D_1 receptors with SKF 38393 often has little or no effect upon motor systems directly, this procedure can markedly alter the animal's responsiveness to D_2 agonists [2, 4, 7, 15, 16]. For instance, SKF 38393 facilitated D_2 responding in quinolinate-lesioned subjects [2], and potentiated D_2-induced stereotyped behaviour in normal animals [7]. Also, when mice were depleted of dopamine with reserpine, injected 3 hr beforehand, it was necessary to stimulate D_1 and D_2 receptors simultaneously before the animals were capable of moving again in anything like a normal fashion [4,16]. In each of these examples, the presence of SKF 38393 enabled the animals to locomote with a greater freedom of movement than was possible with D_2 agonism alone. Although these findings are evidence of positive cooperativity between the two species of dopamine receptor, it would be wrong to assume that D_1/D_2 interactions necessarily result in a mutually amplified motor response.

We first saw signs of behavioural opposition between D_1 and D_2 receptors in non-habituated mice, where the effect of selective D_2 stimulation by RU 24213 was to reduce the incidence of locomotion. Thus low doses of RU 24213 are believed to lower dopaminergic activity and to inhibit locomotion by preferentially activating presynaptic dopamine receptors, whilst higher doses of the drug stimulate postsynaptic dopamine receptors directly and promote stereotypy at the expense of locomotion. In either case we found that the locomotor-reducing action of RU 24213 was strongly counteracted by coadministering SKF 38393 in doses that ordinarily had no motor stimulant action by themselves [15]. The present data provide a rather more bizarre example of D_1 and D_2 receptors working in opposition.

Contrary to expectation, SKF 38393 did not restore motor activity to morphinised rats, nor did it accelerate that induced by RU 24213 [2, 15, 16]. Instead it converted the forward response to RU 24213 into a backward one. This is unusual and has not been observed in other animal models, which means that it probably depends on the presence of morphine. The ability of SCH 23390, a putative D_1 antagonist [5], to block this reversal and to restore forward locomotion, is evidence that the directional influence of SKF 38393 in this sytem is a D_1-mediated phenomenon.

By the same token, the sensitivity of RU 24213 to blockade by metoclopramide indicates D_2 involvement in the ac-

tion of this agonist. On the other hand, that SCH 23390 is also able to annihilate the response to RU 24213 could mean that ongoing stimulation of the D_1 receptor, perhaps by endogenous dopamine, is an essential prerequisite to obtaining a D_2 response in the first place [10]. If this is the case, then the D_1/D_2 balance may be fine one, as overstimulating the D_1 moiety with SKF 38393 results in an entirely different pattern of D_2-mediated behaviour.

Since the speed of walking in the reverse direction generally exceeded that of the animal moving forwards, and because SKF 38393 switched the pattern of orofacial activity from sniffing to licking and chewing, it is clear that D_1 stimulation imposes additional changes on the animal's behaviour in the presence of RU 24213. Interestingly, an increase in the velocity of D_2-induced forward movement, together with the emergence of licking and gnawing similar to that described here for morphinised rats, have also been observed following the coadministration of SKF 38393 and a D_2 agonist to normal [7], quinolinate-lesioned [2] and reserpine-treated animals [16].

Considered together, these findings suggest that certain aspects of the functional relationship that exists between D_1 and D_2 receptors are preserved in the face of other pharmacological treatments that interfere with dopaminergic activity. Exactly how this relationship operates awaits further clarification. All we can say at this stage is that whereas the behavioural consequences of stimulating D_1 receptors in isolation may be unobtrusive, their capacity to alter radically the nature of D_2 motor responding under a variety of experimental conditions emphasises their overall importance in the mechanisms governing voluntary movement.

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