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Circling Evoked by Intranigral SKF 38393: A GABA-Mediated D-1 Response?

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STARR, M. S. AND B. S. STARR. *Circling evoked by intranigral SKF 38393: A GABA-mediated D-1 response?* PHARMACOL BIOCHEM BEHAV **32**(4) 849–851, 1989.—Intranigral injection of microgram doses of the dopamine D-1 receptor agonist SKF 38393, in rats acutely anaesthetised with halothane, did not overtly alter the animals' behaviour. The adenylate cyclase activator forskolin, the GABA uptake inhibitor nipecotic acid and the GABA potentiator pentobarbital, were similarly ineffective when administered singly to one substantia nigra pars reticulata. However, all three treatments interacted synergistically with coinjected SKF 38393 to promote active circling. It is suggested this SKF 38393-induced behavioural response is mediated by GABA released from D-1 receptor-bearing striatonigral neurones.

Substantia nigra Circling SKF 38393 GABA

THE search for a functional role of dopamine D-1 receptors has focussed recently on the substantia nigra pars reticulata (SNR) (4, 10, 13, 14). In this nucleus, the dense population of adenylate cyclase-coupled D-1 receptors appears to reside on the axon terminals of striatonigral GABA-containing neurones, since lesioning these cells virtually eliminates dopamine-stimulated cyclic AMP formation (5,9), D-1-specific binding (3) and GABA (15) from the SNR. Stimulating these D-1 receptors with the selective agonist SKF 38393 (11,12) promotes GABA release in vitro (13) and a weak rotational response of the animal in vivo (1,6). Asin and Montana (1) have speculated, therefore, that the circling induced by activating dopamine D-1 receptors in the SNR may be mediated indirectly by GABA released from striatonigral nerve endings.

To test this hypothesis behaviourally, we microinjected SKF 38393 into one SNR, together with compounds that are known to be capable of increasing the synaptic concentration of GABA (e.g., forskolin, nipecotic acid) (8,13), or GABA's neuroinhibitory action postsynaptically (e.g., sodium pentobarbitone) (17). It was predicted that these treatments would facilitate both GABA neurotransmission and SKF 38393-induced contraversive circling, if GABA neurones were the substrate for this behaviour.

The results show that each drug treatment interacted synergistically with SKF 38393 to enhance circling, which is presented as further evidence of a functional link between dopamine D-1 receptors and GABA activity in the SNR.

METHOD

Animal Treatment

Male Wistar albino rats (Olac) weighing 180–240 g were anaesthetised with 2.5% v/v halothane in oxygen and secured in a Kopf stereotaxic frame. The scalp was incised and a burr hole drilled in the skull. The needle tip of a 5 μl Hamilton syringe (external diameter 0.48 mm) was gradually lowered into the right-hand substantia nigra pars reticulata using the following stereotaxic coordinates: A −4.9, L 2.0 and D 7.5 mm. Bregma and brain surface served as reference points (7).

Vehicle (distilled water) or drug solution (volume 0.5 μl) was expelled over a period of 2 min and the needle left in situ for a further 2 min to allow for diffusion. The needle was then slowly withdrawn, the wound closed and the animal placed singly onto the floor of a circular "open field" (0.85 M diameter, 0.35 M high). Following recovery (5–10 min) each rat was observed for 1 min, every 15 min for 2 hr. Circling was scored as turns/min and consisted of the animal rotating through 360° about the vertical axis.

Maximum circling rates for combined drug treatments were compared with those for water controls and individual drug treatments by the Kruskal-Wallis test. Data for analysis were obtained only from animals in which the correct intranigral placement of injections was confirmed by histology. Following fixation for one week in 4% w/v formaldehyde in saline, brains were frozen and divided into 20 μm coronal sections, which were then stained with Cresyl Violet.

Drugs

SKF 38393 (Research Biochemicals Inc., USA), sodium pentobarbitone (Sigma) and nipecotic acid (Sigma) were dissolved in freshly prepared distilled water. Forskolin (Sigma) was first dissolved in ethanol, then diluted in distilled water.

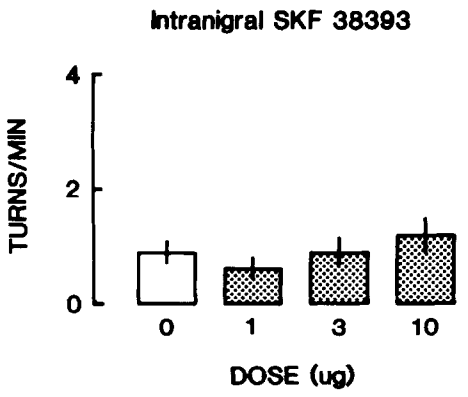

FIG. 1. Contraversive circling induced by unilateral intranigral injection of distilled water (open columns) or SKF 38393 (stippled columns) in rats. All injections were made under acute halothane anaesthesia. Each column is the mean (±SEM) of 10 determinations.

RESULTS

An injection of 0.5 μl distilled water into the right SNR elicited a weak leftward bias of the head and trunk during recovery from anaesthesia. Control animals occasionally described a complete 360° revolution (maximum 0.9+0.2 turns/min, n=10, Fig. 1), which was invariably in the contraversive direction and was easily distinguished from their exploratory perambulations, but there was otherwise no evidence of consistent locomotor asymmetry. The following treatments were similarly ineffective: SKF 38393 (1, 3 and 10 μg, Fig. 1); forskolin (0.1 and 1 μg, Fig. 2); nipecotic acid (1 and 10 μg, Fig. 2); sodium pentobarbitone (0.1 and 1 μg, Fig. 2).

By contrast, administration of 10 μg SKF 38393 (which was behaviourally inactive by itself) in conjunction with the higher (but not the lower) doses of these compounds, gave rise to sporadic yet significant levels of contraversive rotation (Fig. 2); ipsiversive asymmetries were never observed. Whilst engaged in typical exploratory movements, which consisted of walking slowly about the test arena, sniffing the floor and rearing against the walls, rats would stop periodically and execute a deliberate, single, wide circle, before resuming their forward locomotion. This behaviour began 10–15 min postinjection and persisted intermittently throughout the 2 hr of the experiment. There was little evidence of postural asymmetry.

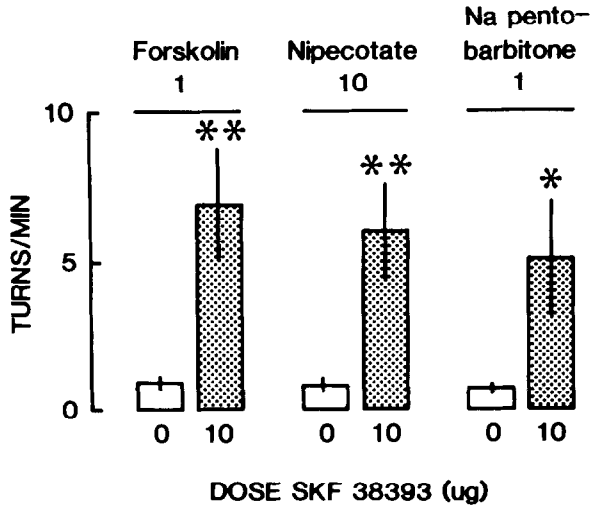

FIG. 2. Effects of enhancing striatonigral GABA neurotransmission on the contraversive circling induced by unilateral intranigral injection of SKF 38393 (stippled columns) in rats. Each column is the mean (±SEM) of 10 determinations. Drug doses given in μg. *$p<0.01$, **$p<0.001$ versus controls (open columns) by Kruskal-Wallis test.

With groups of ten animals, nonparametric analysis revealed a significant interaction occurred between the D-1 agonist and forskolin, $H(3)=18.56$, $p<0.001$, nipecotic acid, $H(3)=17.90$, $p<0.001$, and pentobarbital, $H(3)=11.53$, $p<0.01$.

DISCUSSION

This study has taken advantage of the fact that drugs can enhance GABA neurotransmission in a number of ways, to investigate the theory that GABA released from striatonigral neurones may mediate the behavioural effects of stimulating nigral dopamine D-1 receptors (1, 9, 13). For instance, by activating the enzyme adenylate cyclase, forskolin has been found to increase D-1 receptor-stimulated cyclic AMP synthesis (2) and to enhance the GABA-releasing action of SKF 38393 in potassium-depolarised nigral slices (13). On the other hand, nipecotic acid is held to increase the concentration of GABA at its receptors by reducing the synaptic clearance of the amino acid into neurones (8), while pentobarbital prolongs the opening of chloride ion channels by GABA (17). Though pharmacologically diverse, each of these compounds would be expected to potentiate striatonigral GABA function, and consequently to promote GABA-dependent circling [see (13)]. It is significant, therefore, that all three treatments interacted synergistically with SKF 38393 to make the animals rotate, since this suggests we were witnessing a D-1 receptor-dependent behavioural response that was mediated, at least in part, by GABA.

The direction of this rotational response was invariably away from the injected nigra, which is consistent with the activation of GABA mechanisms within this structure (16). However, in contrast to the robust, nose-to-tail circling evoked by the intranigral administration of a direct GABA receptor agonist such as muscimol (16), that induced by SKF 38393 in the present study was discontinuous and wide, and was not accompanied by postural deviation. This altogether weaker response is perhaps further indicative of the indirect nature of the D-1 receptor-mediated phenomenon.

The extent to which impulse flow in the striatonigral system determines the modulating influence of dopamine D-1 receptors on terminal GABA release is unknown. At this point in time we can only speculate that halothane anaesthesia will have a depressant effect on striatonigral activity, thereby reducing GABA efflux to a point below that capable of sustaining SKF 38393-induced circling. This could explain why microinfusing SKF 38393 by itself into one SNR, gives rise to circling (albeit weak) in conscious, chronically-cannulated rats (1,6), but not in halothane-anaesthetised rats [above and (4,14)].

According to Waldmeier *et al.* (18), the release of endogenous GABA by slices of nigra is not affected by electrical impulses. Thus, it may well be that the only realistic approach for further investigating the regulation of GABA outflow by dopamine D-1 receptors in the SNR, under physiological conditions, will be to monitor GABA release in vivo using dialysis probes.

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