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Rotation Following Intranigral Injections of a Selective D_1 or a Selective D_2 Dopamine Receptor Agonist in Rats

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ASIN, K. E. AND W. E. MONTANA. *Rotation following intranigral injections of a selective D_1 and a selective D_2 dopamine receptor agonist in rats.* PHARMACOL BIOCHEM BEHAV 29(1) 89–92, 1988.—Injections of various nonselective dopamine agonists into the substantia nigra, pars reticulata (SNpr), have been reported to produce contralateral rotation in rats. Since a number of recent dopamine receptor distribution studies have indicated a preponderance of D_1 compared to D_2 dopamine receptor subtypes within the SNpr, we examined the relative behavioral functions of these two subtypes within the nigra by studying rotation following unilateral, local injections of a D_1 (SKF38393) and D_2 (quinpirole) agonist, Significant, dose-dependent contralateral rotation was observed following injections of R,S-SKF38393. This effect was found to be stereoselective to the *R*- enantiomer, suggesting that the effect is receptor mediated. In contrast, quinpirole (LY171555) produced significant, dose-dependent *ipsilateral* rotation following nigral injection. These results suggest that the rotation seen following intranigral injections of nonselective dopamine agonists is due to the stimulation of the D_1 dopamine receptor, and that nigral D_1 and D_2 dopamine receptors may play opposite roles in the control of behavior.

SKF38393 Quinpirole Substantia nigra Rotation D_1 Dopamine receptor D_2 Dopamine receptor

INVESTIGATIONS in the field of receptor pharmacology have indicated the existence of two subtypes of dopamine receptors in brain. Stimulation of the D_1 receptor results in an increase in dopamine-sensitive adenylate cyclase activity, whereas activation of the D_2 receptor either will have no effect or will inhibit enzymatic activity [23].

Behavioral evidence also supports the concept of two distinct dopamine receptor subtypes. The hyperactivity produced by injections of the D_1 agonist SKF38393 into reserpine-pretreated rats can be blocked by the administration of D_1 but not D_2 antagonist drugs, and, conversely, the hyperactivity produced by D_2 agonists can be blocked by D_2 but not D_1 antagonists [2]. Similarly, systemic injections of either SKF38393 or of a D_2 agonist produce contralateral rotation in rats with prior 6-hydroxydopamine (6OHDA) lesions of one nigro-striatal bundle (NSB), and this rotation can be blocked only by D_1 and D_2 antagonists, respectively, or by mixed D_1/D_2 antagonist drugs [3].

A number of studies have examined the distribution of D_1 and D_2 dopamine receptor subtypes within nuclei involved in basal ganglia function. It has been reported that D_1 dopamine receptors are located on striatal projections to the zona reticulata of the substantia nigra (SNzr) [8,22]; indeed, the density of D_1 receptors and proportion of D_1 to D_2 receptors within the SNzr are among the highest in brain, whereas the ratio is much less in the pars compacta (cf. [4, 6–8]). Nigral D_1 dopamine receptors may therefore be importantly involved in basal ganglia function.

Behavioral studies have indicated a role for nigral dopaminergic mechanisms in the control of locomotor activity. Intranigral injections of a number of dopamine agonists have been reported to induce contralateral rotation in rats (cf. [1,10]), but, for the most part, the agonists have all been relatively nonselective for the D_1 or D_2 dopamine receptor subtypes; indeed, little work has been done with regard to the receptor subtypes mediating these effects. In a study examining rotation following intranigral injections of several dopamine agonists, Jackson and Kelly [10] reported that a single dose of SKF38393 induced contralateral rotation in rats. The purpose of this study was to expand on this initial observation and to examine in more detail the relative roles of the D_1 and D_2 dopamine receptors within the SNzr in locomotor activation. We therefore investigated the rotational behavior of rats following unilateral injections of either a selective D_1 agonist, SKF38393 [21], or following injections of a D_2 agonist, quinpirole (the active enantiomer of LY14865) [25]. Our results suggest that the contralateral rotation seen in other studies following intranigral injections of nonselective dopamine agonists is due to stimulation of D_1 dopamine receptors within the SNzr and that the two dopamine receptor subtypes may play different roles in nigral function.

METHOD

Male, Sprague-Dawley rats, approximately 300 g at the time of surgery, served as subjects. On the day of surgery, animals were anesthetized with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. A 22 ga guide cannula, designed to terminate 2 mm above the nigral injection site (AP:3.7 H:2.0, L:2.4 relative to the intraaural line) [20] was affixed to the skull with dental acrylic, and a 28 ga

stainless steel obdurator, the length of the guide cannula, was screwed into the outer cannula. Following at least a one week recovery period, testing for rotation was begun. Rats were individually placed in round, opaque tubs (30 cm dia) in a room equipped with a white noise generator for 15 min prior to intranigral injections. After the habituation period, animals were lightly restrained, and a 28 ga injection cannula, which was trimmed to terminate within the SNzr, was inserted through the guide cannula. Drugs were dissolved in sterile water and were injected in a volume of 1 μl over a 1 minute period; the injection cannula was left in situ for an additional 30 sec before removal. Following the injection, the stainless steel obdurator was replaced in the guide cannula and the animal was returned to the rotometer. Beginning 4 min later, the number of complete rotations was counted by the experimenters for 15 min. At least 3 days separated each test, and drug treatments were administered in a random order. Following behavioral testing, rats were perfused transcardially under deep anesthesia with a 10% formalin solution and the brain was removed and fixed in the solution for at least 5 days. The brain was then frozen and sixty-four micron sections were taken through the extent of the implants. Following staining with cresyl violet, sections were examined for nigral injection site and for degree of nonspecific damage without reference to the behavioral data of the animal.

In the first experiment, rotation following intranigral injections of R,S-SKF38393 (Research Biochemicals, Natick, MA) (0, 1.25, 5.0 or 10 μg) was examined. In a second experiment we examined the stereospecificity of the drug-induced response by comparing the rotation produced by injections of either vehicle or 5 μg R- or S-SKF38393. In the third experiment, we examined the behavior of rats given unilateral nigral injections of the D_2 agonist quinpirole (Eli Lilly, Indianapolis, IN) (0, 0.03, 0.125 or 0.25 μg). Rats were initially tested for rotation following an intranigral injection of 10 μg SKF38393 and only those rats demonstrating at least 20 total revolutions were tested further. Different groups of animals were used in each experiment.

Rotational data for the two dose-response studies using SKF38393 or quinpirole were analyzed using a one-way ANOVA with repeated measures. Post-hoc comparisons were made using the Newman-Keuls test. Rotation following injections of R- or S-SKF38393 vs. vehicle were analyzed using the paired t-statistic.

RESULTS

Cannula Placements

Examination of stained sections indicated that in some animals either the injection site was outside of the substantia nigra pars reticulata or that substantial nonspecific damage was produced by the injection cannula and/or the injection itself. For all studies, only the data from those animals with correct cannula placements terminating within the reticulata, and with minimal nonspecific damage (i.e., little or no gliosis or necrosis at the injection site) were included in the analyses. Due to our strict criteria, the data from approximately 40% of the animals were eliminated on histological grounds; at no time was reference made to the behavioral responsiveness of the animals during examination of brain tissue.

Intranigral Injections of R,S-SKF38393 and Enantiomers

Rotation following intranigral administration of various doses of R,S-SKF38393 (N=9) may be seen in Fig. 1. Injec-

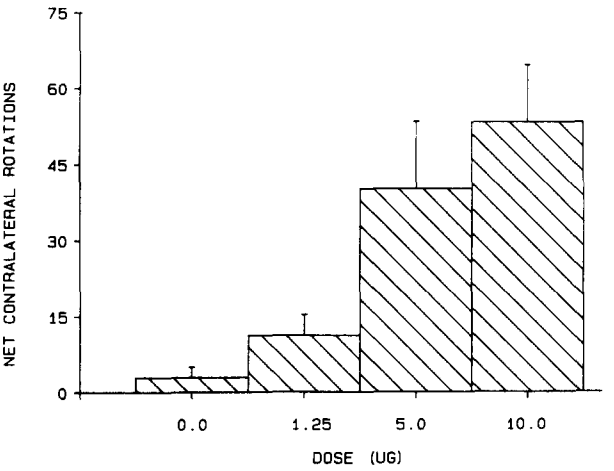

FIG. 1. Mean (±sem) net total number of rotations following injections of vehicle or various doses of R,S-SKF38393 into the substantia nigra pars reticulata. Rotations were counted over a 15 min period beginning 4 min after drug injection.

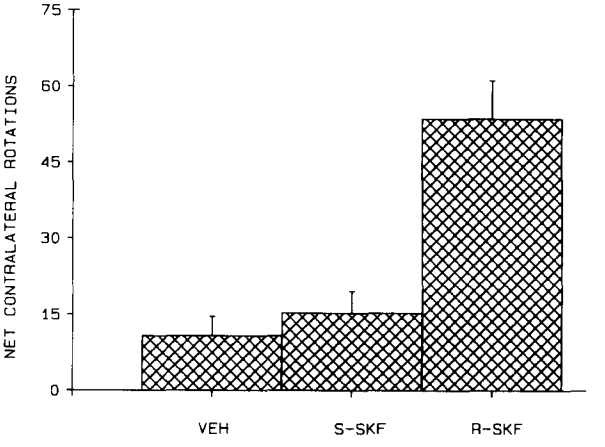

FIG. 2. Mean (±sem) net total number of contralateral rotations over a 15 min period 4 min following injections of either vehicle or 5 μg of R-SKF38393 or S-SKF38393 into the substantia nigra.

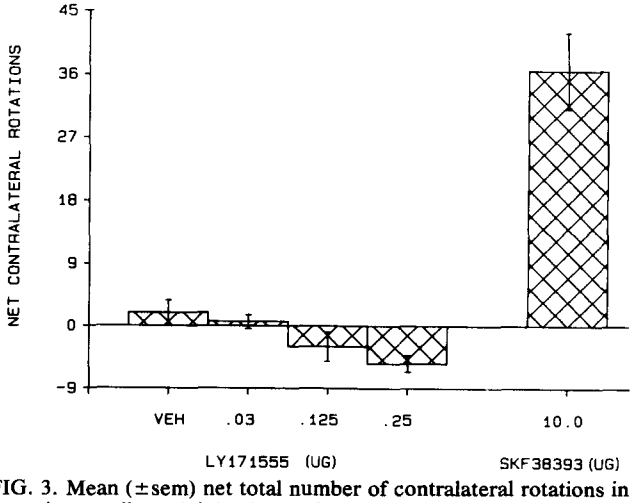

FIG. 3. Mean (±sem) net total number of contralateral rotations in rats given unilateral injections of vehicle or various dosages of quinpirole. At the right of the figure is indicated the mean number of contralateral rotations demonstrated by these same animals in response to 10 μg R,S-SKF38393.

tions of this compound resulted in significant, $F(3,24)=9.93$, $p<0.005$, levels of contralateral rotation during the 15 min measurement period and post-hoc comparisons indicated that significant degrees of rotation were seen following injections of the two highest doses ($p<0.01$). The stereospecificity of this effect of SKF38393 was demonstrated in the second experiment (Fig. 2). Intranigral injections of S-SKF38393 (5 μg) (N=4) failed to produce significant contralateral rotation compared to vehicle, $t(3)<1$, whereas injections of R-SKF38393 induced significant levels of rotation, $t(3)=7.84$, $p<0.01$.

Intranigral Injections of Quinpirole

In contrast to the contralateral rotation seen after administration of SKF38393, injections of the D_2 agonist quinpirole resulted in significant *ipsilateral* rotation, $F(3,27)=7.232$, $p<0.005$ (N=10). These animals demonstrated contralateral rotation in response to R,S-SKF38393 [mean (±sem) number of rotations= 36.4±5.36]. The results of this experiment may be seen in Fig. 3. Post hoc analysis indicated significant levels of ipsilateral rotation following injections of the 0.12 or 0.25 μg dosages ($p<0.05$) of quinpirole. The absolute magnitude of the rotation produced by quinpirole, even at the highest dose, was below that produced by the D_1 agonist. However, results of pilot studies indicated that injections of higher doses (i.e., 1 or 5 μg) produced marked sedation.

DISCUSSION

The present study was concerned with investigating and comparing the behavioral effects of selective, unilateral stimulation of the D_1 and D_2 dopamine receptor subtypes within the SNzr. With regard to stimulation of the D_1 receptor, our results indicate that unilateral injections of the D_1 agonist SKF38393 can induce contralateral rotation in a dose-dependent manner and that the effect is stereoselective. Our finding that significant levels of rotation are seen following the R-, but not S-, enantiomer is consistent with the results of other behavioral studies [17] and suggests that the effect of this compound on rotation is receptor mediated. Binding studies indicate that only R-SKF38393 has nanomolar affinity for the D_1 (^{3}H-piflutixol) binding site [18].

The present study is the first to provide behavioral evidence that some functions of D_2 receptors within the SNzr may be opposite to those of the D_1 receptor since the direction of rotation following injections of quinpirole was ipsilateral whereas that produced by SKF38393 was contralateral. Although the degree of rotation in response to nigral injections of quinpirole was small, it was statistically significant at the 0.005 level of probability and was opposite in direction to that produced by SKF38393. Neurochemical studies have indicated opposite effects of D_1 and D_2 agonists on cyclic AMP efflux and accumulation within the striatum [11,24], but our study is the first to demonstrate opposite behavioral effects of D_1 and D_2 agonists after their direct application into brain.

It is possible that the ipsilateral direction of rotation produced by quinpirole reflects interactions of this compound with receptors located on the dendrites of dopaminergic compacta cells extending into the SNzr and which are probably of the D_2 type [15]. The resulting inhibition of these cells might then be expected to produce ipsilateral rotation; the relatively small magnitude of the effect might reflect the "basal" release of dopamine within the contralateral striatum. The contralateral rotation which has been reported following systemic injections of D_2 agonists in rats with unilateral 6OHDA NSB lesions (cf. [3]) most likely reflects the post-synaptic actions of these compounds within the striatum. Contrary to the effects of D_2 agonists, intranigral injections of SKF38393 might be expected to interact with the D_1 receptors on striatonigral efferents. It is unlikely that subtle differences in cannula placements can account for the differential behavioral effects of the two test compounds since rats used in the D_2 agonist study were first tested for rotation following injections of SKF38393. Our results also demonstrate that it is possible to obtain locomotor activation in neurologically intact rats in response to SKF38393 without first allowing the animals a long period to habituate to the testing apparatus [16].

Several lines of evidence indicate a physiologic role for the dopamine receptors within the SN. Dendritic release of dopamine by nigral compacta cells in response to electrical or pharmacologic stimuli has been demonstrated and is capable of altering striatal dopamine mechanisms [5, 9, 14, 19]. Behavioral data also support a role for nigral dopamine receptors. Dopamine, ADTN, ±-3-PPP, ergometrine, apomorphine and amphetamine have all been reported to produce contraversive circling following unilateral nigral (pars reticulata) injection [1, 10, 12, 13]. Our results suggest that the rotation produced by these compounds may be mediated through direct activation of D_1 dopamine receptors within the reticulata.

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REFERENCES

1. Andrews, C. D. and G. N. Woodruff. Turning behavior following nigral injections of dopamine agonists and glycine. *Eur J Pharmacol* **84:** 169–175, 1982.
2. Arnt, J. Behavioral stimulation is induced by separate dopamine D-1 and D-2 receptor sites in reserpine-pretreated but not in normal rats. *Eur J Pharmacol* **113:** 79–88, 1985.
3. Arnt, J. and J. Hyttel. Differential inhibition by dopamine D-1 and D-2 antagonists of circling behavior induced by dopamine agonists in rats with unilateral 6-hydroxydopamine lesions. *Fed J Pharmacol* **102:** 349–354, 1984.
4. Boyson, S. J., P. McGonigle and P. B. Molinoff. Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. *J Neurosci* **6:** 3177–3188, 1986.
5. Cheramy, A., V. Leviel and J. Glowinski. Dendritic release of dopamine in the substantia nigra. *Nature* **289:** 537–542, 1981.
6. Dawson, T. M., P. Barone, A. Sidhu, J. K. Wamsley and T. N. Chase. Quantitative autoradiographic localization of D-1 dopamine receptors in the rat brain: Use of the iodinated ligand (^{125}I)SCH23982. *Neurosci Lett* **68:** 261–266, 1986.
7. Dawson, T. M., D. R. Gehlert, R. T. McCabe, A. Barnett and J. K. Wamsley. D-1 dopamine receptors in the rat brain: Quantitative autoradiographic analysis. *J Neurosci* **6:** 2352–2365, 1986.

8. Dubois, A., M. Savasta, O. Curet and B. Scatton. Autoradiographic distribution of the D1 agonist (^{3}H)SK&F38393, in the rat brain and spinal cord. Comparison with the distribution of D2 dopamine receptors. *Neuroscience* **19:** 125–137, 1986.
9. Geffen, L. B., T. M. Jessell, A. C. Cuello and L. L. Iverson. Release of dopamine from dendrites in rat substantia nigra. *Nature* **260:** 258–260, 1976.
10. Jackson, E. A. and P. H. Kelly. Nigral dopaminergic mechanisms in drug-induced circling. *Brain Res Bull* **11:** 605–611, 1983.
11. Kelly, E. and S. R. Nahorski. Specific inhibition of dopamine D1-mediated cyclic AMP formation by dopamine D2, muscarinic cholinergic and opiate receptor stimulation in rat striatal slices. *J Neurochem* **47:** 1512–1516, 1986.
12. Kelly, E., P. Jenner and C. D. Marsden. Behavioural effects mediated by unilateral nigral dopamine receptor stimulation the rat. *Exp Brain Res* **55:** 243–252, 1984.
13. Kelly, E., P. Jenner and C. D. Marsden. Lack of effect of intranigral administration of a dopamine analogue, (±-3-(3-hydroxyphenyl)-N-n-propylpiperidine (±-3-PPP), on nigrostriatal dopamine neurons. *Neurosci Lett* **56:** 57–62, 1985.
14. Leviel, V., A. Cheramy and J. Glowinski. Role of the dendritic release of dopamine in the reciprocal control of the two nigrostriatal dopaminergic pathways. *Nature* **280:** 236–239, 1979.
15. Matthews, R. T. and D. C. German. Evidence for a functional role of dopamine type-1 (D1) receptors in the substantia nigra of rats. *Eur J Pharmacol* **120:** 87–93, 1986.
16. Molloy, A. G. and J. L. Waddington. Sniffing, rearing and locomotor responses to the D-1 dopamine receptor agonist R-SK&F38393 and to apomorphine: Differential interactions with the selective D-1 and D-2 antagonists SCH23390 and metoclopramide. *Eur J Pharmacol* **108:** 305–308, 1985.
17. Molloy, A. G. and J. L. Waddington. Dopaminergic behavior stereospecifically promoted by the D1 agonist R-SK&F38393 and selectively blocked by the D1 antagonist SCH23390. *Psychopharmacology (Berlin)* **82:** 409–410, 1984.
18. Molloy, A. G., K. M. O'Boyle, M. T. Pugh and J. L. Waddington. Locomotor behaviors in response to new selective D-1 and D-2 dopamine receptor agonists, and the influence of selective antagonists. *Pharmacol Biochem Behav* **25:** 249–253, 1986.
19. Paden, C., C. J. Wilson and P. M. Groves. Amphetamine-induced release of dopamine from the substantia nigra in vitro. *Life Sci* **19:** 1499–1506, 1976.
20. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press, 1982.
21. Setler, P. E., H. M. Sarau, C. L. Zirkle and H. L. Saunders. The central effects of a novel dopamine agonist. *Eur J Pharmacol* **50:** 419–430, 1978.
22. Spano, P. F., M. Trabucchi and G. DiChiara. Localization of nigral dopamine-sensitive adenylate cyclase on neurons originating from the corpus striatum. *Science* **196:** 1343–1345, 1977.
23. Stoof, J. C. and J. W. Kebabian. Opposing roles for D1 and D2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. *Nature* **294:** 366–368, 1981.
24. Stoof, J. C. and P. F. H. M. Verheijden. D-2 receptors stimulation inhibits cyclic AMP formation brought about by D-1 receptor stimulation in rat neostriatum but not nucleus accumbens. *Eur J Pharmacol* **129:** 205–206, 1986.
25. Tsuruta, K., E. A. Frey, C. W. Grewe, T. E. Cote, R. I. Eskay and J. W. Kebabian. Evidence that LY141865 specifically stimulates the D2 dopamine receptor. *Nature* **292:** 463–465, 1981.