Pharmacology Biochemistry & Behavior, Vol. 21, pp. 73–77, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Opposite Locomotor Asymmetries Elicited From the Medial and Lateral SN by Modulation of SN DA Receptors

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VACCARINO, F. J. AND K. B. J. FRANKLIN. *Opposite locomotor asymmetries elicited from the medial and lateral SN by modulation of SN DA receptors.* PHARMACOL BIOCHEM BEHAV **21**(1) 73–77, 1984.—In combination with systemic d-amphetamine (1 mg/kg), apomorphine or alpha-flupenthixol were unilaterally injected into either the medial or lateral substantia nigra pars compacta (SNC) of adult male hooded rats. Direction and magnitude of circling were measured. Alpha-flupenthixol (5 and 15 μg) injected into the medial SNC caused rats to circle contraversive to the injected side while laterally placed injections produced ipsiversive circling. Apomorphine (5 and 15 μg) induced ipsiversive circling when injected into the medial SNC but had no effect in the lateral SNC. The results show that nigrostriatal mediated circling can be induced by modulation of dopamine (DA) receptors in the SNC. Furthermore, the findings support the notion that lateral SNC DA neurons are functionally antagonistic to medial SNC DA neurons with respect to circling. The results also suggest that there may be medial-lateral SNC differences in the type of DA receptors.

Dopamine Autoreceptors Locomotor asymmetries Circling Substantia nigra

WHEN animals with unilateral lesions of the nigrostriatal pathway are given dopamine (DA) agonists they are generally found to circle away from the striatum with the most DA activity (contraversive circling) [12,26]. Thus, systemic amphetamine treatment induces circling towards the lesioned side (ipsiversive circling) presumably because amphetamine preferentially releases dopamine from nigrostriatal DA terminals on the intact side. On the other hand, apomorphine induces contraversive circling which is thought to depend on activation of supersensitive striatal dopamine (DA) receptors on the lesioned side [12,26]. Direct chemical and electrical stimulation studies also support the notion that rats circle away from the more active nigrostriatal system. In unlesioned rats both electrical and chemical stimulation of the nigrostriatal DA system will produce contraversive circling [1, 3, 12, 14, 18].

Recent evidence, however, suggests that the nigrostriatal dopamine system is not functionally homogeneous with respect to circling [25, 27, 28]. Consistent with earlier results, it was found that in rats with unilateral lesions of the medial SN amphetamine treatment caused ipsiversive circling. However when the lesions were restricted to the lateral parts of the SN amphetamine produced contraversive circling [27]. In addition, electrical stimulation of the medial substantia nigra pars compacta (SNC) was found to produce contraversive circling while stimulation of the lateral parts of the SNC produced ipsiversive circling [28]. Both ipsi- and contraversive stimulation-induced circling were dose-dependently blocked by the DA antagonist, pimozide [28]. These results were taken to suggest that circling in both directions can be driven by DA neurons on one side, implying that DA neurons in the lateral SN are functionally opposed to those in the medial SN.

The SN contains DA receptors which when stimulated inhibit the impulse flow of DA neurons [4, 5, 22, 24]. It would therefore be predicted that direct unilateral application of a DA agonist into the medial SNC would selectively depress the firing of medial SNC DA neurons and cause ipsiversive circling. Conversely, direct application of a DA antagonist into the medial SNC would be expected to increase the firing rate of medial SNC neurons to produce contraversive circling. In light of the medial-lateral SN differences reviewed above, opposite behavioral effects would be expected in the lateral SN. That is, direct application of a DA agonist into the lateral SNC should produce contraversive circling while a DA antagonist should produce ipsiversive circling.

The present experiments tested these predictions by investigating the effects of the DA agonist apomorphine [16,21] and the DA antagonist alpha-flupenthixol [7, 13, 21] microinjected into the medial or lateral portions of the SNC. In previous studies [27,28] we observed that the circling elicited from stimulation of DA cells resembled normal locomotion with a bias to one direction. Microinjections of drugs into the SN produce very little locomotion and hence very low cir-

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cling scores (F. Vaccarino, unpublished PhD thesis). To enhance circling in the experiments reported here, animals were injected with amphetamine IP to increase locomotion.

METHOD

Subjects

Subjects were 52 male Long Evans hooded rats obtained from Charles River Canada Inc. They were housed, in 14×25×47 cm plastic cages on a 12-hr diurnal cycle. Food (Purina Rat Lab Chow) and water were available ad lib throughout the experiment. The rats weighed 250–350 g at the time of electrode implantation.

Apparatus

Circling was recorded in an inexpensive apparatus designed and constructed in our laboratory. The rat was placed within a galvanized metal cylinder (29 cm in diameter) which sat on top of a wooden platform. A velcro harness, wrapped around the rat's abdomen, was attached to a mercury commutator (Mercotac) via an 8-gauge wire. Thus any circling by the rat resulted in an equivalent rotation of the commutator. A surgical thread was attached to the commutator so that the rotation of the commutator resulted in the thread winding around the commutator shaft. The distal end of the thread, after passage through several metal eye screws attached to the ceiling, was attached to a light weight which hung several centimeters above the floor. Thus, any circling by the rat resulted in a winding of the thread and an upward progression of the weight. The net circling was determined by the distance the weight moved up a calibrated wall. The preferred direction of circling was determined by the direction the thread was wound around the commutator.

Surgery

Under sodium pentobarbital anesthesia (50 mg/kg) a 23-gauge stainless steel guide cannula (Plastic Products Ltd.) was implanted in the right hemisphere of each rat. The tips of the guide cannulas were aimed 1 mm dorsal of the medial or lateral SNC. The coordinates for the medial group were: 3.2 mm posterior to bregma, 1.9 mm lateral to the midline suture and 7.5 mm ventral to the dorsal surface of the skull. Coordinates for the lateral group were: 3.2 mm posterior to bregma, 2.6 mm lateral and 6.8 mm ventral. The nose bar was set 5 mm above the interaural line. A 31-gauge dummy cannula was inserted into the guide cannula so that the base was flush with the guide cannula base. Injection cannulas (31-gauge, Plastic Products Ltd.) protruded 1 mm ventral of the guide cannula tip.

Procedure

Testing began ten days following surgery. On the first test day rats were placed in the circling apparatus for 1 hr, drug free, in order to habituate to the apparatus. On days 2, 4, and 6, rats were tested under one of 3 drug conditions: saline vehicle, 5 μg alpha-flupenthixol or 15 μg apomorphine, and 15 μg alpha-flupenthixol or 25 μg apomorphine. These drugs were microinjected into the SNC in a volume of 0.5 μl injected over 30 sec. Each rat received drugs in a different random order. A total of 22 rats received alpha-flupenthixol and 30 rats received apomorphine. In addition to the microinjection each rat received an IP injection of 1 mg/kg d-amphetamine (1 ml/kg) volume). Immediately following drug administration each rat was placed in the circling apparatus for 1 hr. For statistical analyses, ipsiversive circling was expressed as a negative integer and contraversive circling was expressed as a positive integer.

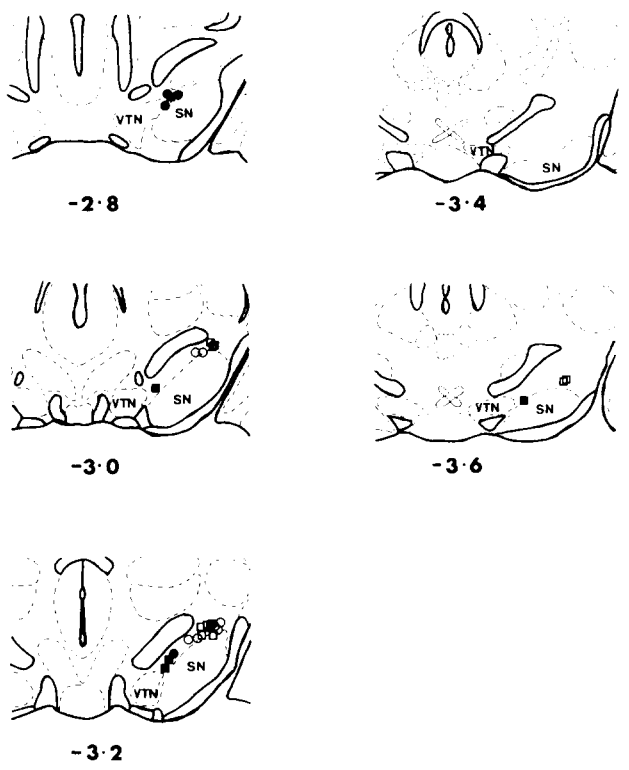

FIG. 1. Apomorphine (square symbol) and alpha-flupenthixol (circles) microinjection sites in the medial SN (shaded symbols) and lateral SN (open symbols). Numbers below each section represent the AP location relative to Bregma (mm). SN: substantia nigra; VTN: ventral tegmental nucleus.

Histological Procedure

Following testing, rats were anaesthetized with sodium pentobarbital (100 mg/kg) and perfused first with 0.9% saline and then with 10% formalin. The brains were removed and kept in 10% formalin for 2 days. Forty μm frozen sections were cut and stained with thionin for microscopic examination.

RESULTS

Alpha-Flupenthixol

Eight of the 22 rats tested had cannula tips dorsal to the SN and were not included in the analyses. None of these rats circled. Twelve rats tested with alpha-flupenthixol circled. Four of these rats had injection cannula tips in the medial portion of the SNC and 7 had tips in the lateral portion of the SNC (see Fig. 1). The brain of one medial circler was lost and the tip location could, therefore, not be identified.

Analysis of variance comparing injection site × drug revealed a significant interaction, $F(2,20)=4.30$, $p<0.028$. Across group comparisons showed that after saline microinjections circling direction did not differ between the 2

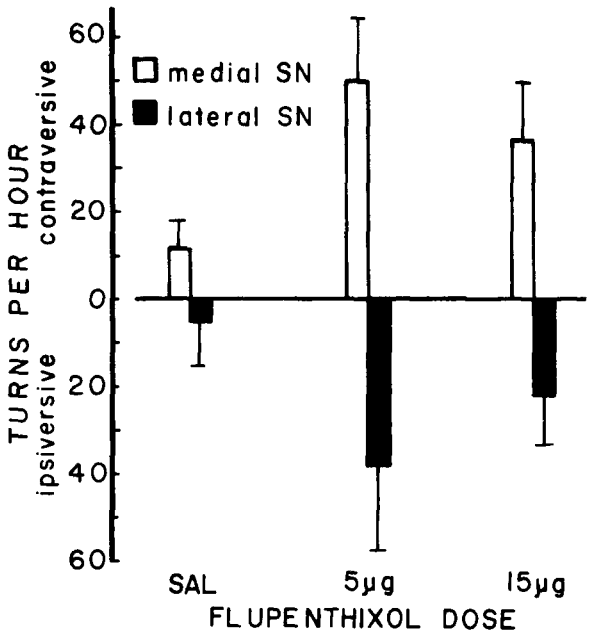

FIG. 2. Magnitude and direction of circling following saline and alpha-flupenthixol microinjections into the medial and lateral SN. Vertical bars represent standard errors.

groups, $t(10)=1.1$, NS, ts calculated using the mean square error from the analysis of variance. After alpha-flupenthixol microinjection the two groups circled in opposite directions at the 5 μg dose, $t(10)=5.1$, $p<0.05$, and at the 15 μg dose, $t(10)=4.0$, $p<0.05$. It can be seen in Fig. 2 that medial rats circled contraversively with alpha-flupenthixol while rats with lateral injections circled ipsiversively.

Apomorphine

Of the 30 rats tested with apomorphine 5 had cannula tips in the medial portion of the SNC and 11 had cannula tips in the lateral portion of the SNC (see Fig. 1). The remaining rats had cannula tips dorsal to the SNC and were not included in the analysis. None of these latter rats circled.

Analysis of variance comparing group × drug revealed a significant group effect, $F(1,14)=14.19$, $p<0.0021$. It can be seen in Fig. 3 that after injections in the medial SNC rats circled ipsiversively while with lateral injections rats showed no circling. Across group comparisons showed that the medial group circled more than the lateral group in all three drug conditions: saline, $t(14)=2.3$, $p<0.05$; 15 μg apomorphine, $t(14)=4.1$, $p<0.05$; 25 μg apomorphine, $t(14)=3.4$, $p<0.05$. Within group comparisons showed that medial rats circled significantly more with both 15 μg apomorphine, $t(8)=4.8$, $p<0.05$ and 25 μg apomorphine, $t(8)=2.8$, $p<0.05$, than with saline. It may be noticed in Figs. 2 and 3 that with saline rats tend to circle in the same direction as on drug trials. The raw data suggests that this is a consequence of the randomized order of testing. When saline was received first in the series there was no bias. After 1 or 2 drug injections with evoked circling rats tended to circle in the same direction on saline trials. This may indicate conditioning of the drug effect to the test situation such as has been observed with other dopaminergic drugs [31].

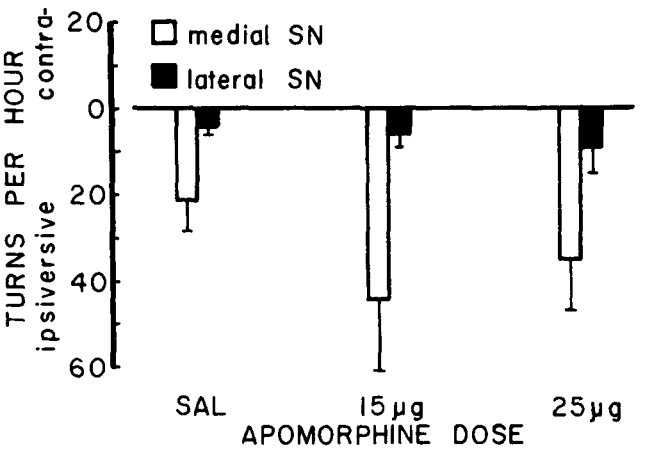

FIG. 3. Magnitude and direction of circling following saline and apomorphine microinjections into the medial and lateral SN. Vertical bars represent standard errors.

DISCUSSION

As would be expected from the original circling model [26], the DA agonist apomorphine produced ipsiversive circling when injected unilaterally into the medial SNC. Presumably, this reflects a depression of medial SNC DA activity caused by stimulation of medial SNC DA receptors, which results in an imbalance in striatal DA activity favoring the non-injected side. Consistent with this interpretation, the DA antagonist alpha-flupenthixol produced contraversive circling when injected into the medial SNC. This reflects an increase in medial SNC DA activity on the injected side caused by blocking DA receptors in that region.

As predicted, a different pattern was seen following lateral SNC injections. Alpha-flupenthixol produced ipsiversive circling when injected into the lateral SNC which was opposite to the direction of circling produced by such injections in the medial SNC. This is interpreted to reflect an increase in activity of lateral SNC DA neurons which are functionally opposed to medial SNC DA neurons. The opposite effects expected from stimulation of lateral receptors were not observed. Apomorphine had no effect in the lateral SNC.

Previous studies investigating the behavioral effects of direct DA agonists and antagonists into the SN have not been consistent. One recent study showed that apomorphine microinjections (5 μg) into the medial SNC of mice resulted in weak ipsiversive circling [19]. These results are in agreement with ours and were viewed as consistent with autoregulatory inhibition of impulse flow in DA neurons. On the other hand, Arnt and Scheel-Kruger reported that unilateral injections of apomorphine into the SNC (10 μg), in combination with peripheral apomorphine (0.5 mg/kg, SC), produced a weak contraversive locomotor bias [2]. Since their SNC injection sites appear to correspond to our medial SNC sites these results do not agree with ours. A possible explanation for this discrepancy may lie in the different methods used in the two studies. Arnt and Scheel-Kruger injected water into the SN contralateral to the apomorphine injection and used apomorphine as a peripheral stimulant. These treatments may have modified the imbalance in DA release expected from unilateral apomorphine application. For instance, small differences in DA neuron impulses flow

between the 2 sides of the brain may have been obscured postsynaptically by apomorphine's indiscriminate bilateral stimulation of postsynaptic DA receptors.

At low doses in vivo, amphetamine increases the impulse dependent release of DA [10, 22, 23, 30], and depresses DA cell firing [4]. Intranigral flupenthixol should antagonize amphetamine induced depression of DA cell firing [4] and therefore exaggerate amphetamine stimulation of DA release. By similar reasoning apomorphine should increase the amphetamine-induced depression of DA cell firing and reduce amphetamine stimulation of DA release. Amphetamine also increases locomotion by its effect on mesolimbic DA projections [15]. Thus, in the present experiments the effect of systemic amphetamine would be to exaggerate circling but not alter the direction determined by the microinjections.

From our results it is not possible to identify the DA receptors involved. DA cell firing may be inhibited by activation of either D2-autoreceptors on DA cell bodies or dendrites [4,7] or by DA sensitive adenylate cyclase-linked receptors (D1) on striatonigral terminals [7,22]. Flupenthixol probably binds at both D1 and D2 receptors while apomorphine has higher affinity and efficacy at the D2 receptor [7]. In light of this the fact that we did not elicit circling with apomorphine in the lateral SN might suggest that the lateral SN receptors are of the D1 type. However, activation of either receptor type (D1 or D2) would have the same effect on DA cell firing. Thus, the medial lateral differences observed here cannot be accounted for by actions on different receptor types. Our finding of opposite directions of circling elicited from different parts of the SN is consistent with our hypothesis that there exist functionally opposed subsets of nigrostriatal DA neurons.

There are also DA receptors on nigral non-DA efferents [5,7] which could be affected by our treatments. It might be argued that since there are fewer DA cells in the lateral SNC our lateral injections primarily affect non-DA efferents in the pars reticulata and thus produces circling opposite to medial injections. However, the probable pathways involved in such as effect are inconsistent with this possibility. Interference with the striatonigral GABA pathway by lesions [11] or GABA antagonists [8] produces ipsiversive circling. Since GABA is inhibitory it can be inferred that inhibition of SN non-DA efferents causes contraversive circling. This interpretation is confirmed by the fact that kainate lesions of the pars reticulata produce spontaneous contraversive circling [8]. Since dopamine's action on these efferents is excitatory [20] flupenthixol would be expected to reduce activity in non-DA efferents and cause contraversive circling. In fact, flupenthixol in the lateral SNC produced ipsiversive circling as predicted.

The results of the present study confirm and extend previous findings suggesting that lateral SN DA neurons are functionally opposed to medial SN DA neurons [27,28]. However, the mechanisms underlying these differences are not yet understood. One possibility is that DA released from different parts of the nigrostriatal projection activates either inhibition-mediating or excitation-mediating DA receptors which, in the cat, are localized in different striatal regions [6]. However, direct evidence for such a distinction in DA receptors in the rat is lacking. While both medial and lateral SN lesions do increase (^{3}H)-spiroperidol binding in the striatum no regional differences in the characteristics of the binding have been detected [25]. Another hypothesis is suggested by the fact that the topography of the SNC is preserved in the projection to the striatum [9,17]. Medial and lateral DA neurons project to different parts of the striatum which may in turn project to different motor output stations. In favour of this possibility we have recently observed that unilateral lesions in the region of the superior colliculus block the contraversive circling elicited by alpha flupenthixol microinjected into the medial SN but not the ipsiversive circling elicited by alpha flupenthixol in the lateral SN [29].

ACKNOWLEDGEMENTS

This research was supported by NSERC Grant No. 6303 to K. Franklin and a grant from the Parkinson Foundation of Canada. F. Vaccarino was supported by an NSERC scholarship. We thank Smith, Kline and French (Canada) Ltd. for their gift of d-amphetamine. Jim Blackburn assisted in running the experiments.

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