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Circling Produced by Serotonin and Dopamine Agonists in Raphe Lesioned Rats: A Serotonin Model

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SLATER, P. *Circling produced by serotonin and dopamine agonists in raphe lesioned rats: A serotonin model.* PHARMAC. BIOCHEM. BEHAV. 13(6) 817–821, 1980.—The serotonin (5-HT) receptor agonists quipazine and 5-methoxy-N,N-dimethyltryptamine caused slow, contralateral circling, which was not dose-related, when given to rats with an asymmetric, electrolytic lesion of the midbrain medial raphe nucleus. No circling was recorded after administration of fenfluramine, a compound that releases 5-HT. The dopamine drugs apomorphine and d-amphetamine caused vigorous circling in raphe lesioned rats which was increased further by quipazine but antagonized by fenfluramine. The effects of the 5-HT drugs on d-amphetamine circling were closely dose-related. The results suggest that modulation of d-amphetamine circling by 5-HT agonists in rats with a specific, asymmetric 5-HT lesion could provide the basis for a 5-HT rotation model.

5-HT agonists Raphe lesion Apomorphine d-Amphetamine Rotation

THE rat with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigro-striatal dopamine neurons [21] is used extensively as a model for studying striatal dopamine function in vivo. The basis of the model is that dopamine agonists cause rapid, dose-dependent circling which varies in direction according to whether the agonist used has a pre- or postsynaptic site of action. Compounds that directly stimulate dopamine receptors, such as L-DOPA and apomorpine, produce contraversive circling when the dopamine receptors in the lesioned striatum develop denervation supersensitivity. On the other hand, compounds such as d-amphetamine will cause ipsiversive circling by releasing more endogenous dopamine from the intact striatum [8].

There is a need for equally convenient behavioural models to study other striatal neurotransmitters in vivo. A circling model, similar to that used for dopamine, has been suggested for serotonin (5-HT) [10]. This model relies on the principle that rats with a unilateral lesion of the striatal 5-HT innervation, circle contralaterally when given 5-HT agonists or the 5-HT precursor 5-hydroxytryptophan [7,10]. This however makes a somewhat unsatisfactory model because the circling produced by 5-HT agonists is very slow and therefore difficult to distinguish from the spontaneous turning [14]. There are also reported inconsistencies in the direction of the circling induced by 5-HT agonists [10, 14, 22]. 5-HT lesions are most often made by injecting neurotoxic compounds such as 5,6-dihydroxytryptamine [4] into the medial forebrain bundle. This does not, however, produce a specific 5-HT lesion because it also depletes forebrain dopamine [22].

A close functional relationship exists between 5-HT and dopamine in the striatum [6]. Rats with striatal 5-HT lesions show rotational responses to dopamine agonists [6, 7, 14, 22] and activation of 5-HT receptors alters striatal dopamine release [17]. The present experiments have examined the effects of compounds that directly stimulate 5-HT receptors and release endogenous 5-HT on dopamine mediated circling behaviour in rats with a specific 5-HT lesion made by lesioning the midbrain raphe nucleus [7,14].

METHOD

Female Sprague-Dawley rats (150–160 g) were anaesthetized with methohexitone sodium (Brietal, 50 mg/kg IP) and positioned in a stereotaxic frame. Electrolytic lesions were made using an insulated tungsten electrode with a 0.5 mm exposed tip. A DC current (1 mA; 15 sec) was supplied by a Grass S88 stimulator and constant current unit. The return current passed through the ear bars. Small asymmetric lesions of the medial raphe nucleus were made by lowering the electrode at a 20° angle from the vertical to the coordinates A 0.3 mm; L 0 mm; H −3.3 mm. The coordinates were based upon, but slightly modified from the atlas of König and Klippel [10].

The rats were observed for spontaneous and drug-induced circling following a 14 day post-operative interval. Each rat was placed on a flat surface in an open field and the number of complete 360° turns was counted by an observer who was unaware of the treatment schedule. Spontaneous circling was recorded 20 min after the rats had been introduced to the open field. Lesioned rats were randomly assigned to groups. Drug-induced circling was initiated by IP administration of dopamine and 5-HT agonists. The rats were immediately returned to the open field. Control (saline treated) circling rates were first established and then each group of rats received several challenges with different doses of a drug with a recovery interval of at least 2 days between. Control circling rates were redetermined after 10 days.

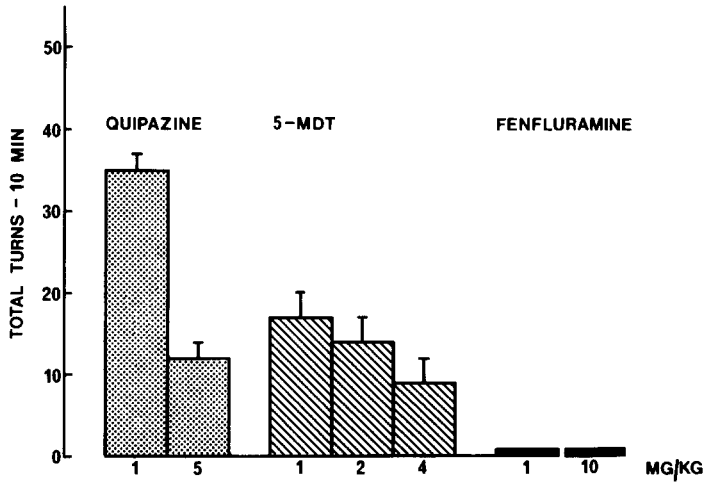

FIG. 1. The contralateral circling produced by quipazine (1 and 5 mg/kg IP, 10–20 min), 5-methoxy-N,N-dimethyltryptamine (5-MDT, 1-4 mg/kg IP, 10–20 min) and fenfluramine (1 and 10 mg/kg IP, 20–30 min) in rats with an asymmetric lesion of the medial raphe nucleus. Each result is the mean obtained with 8 rats. Vertical bars represent SEM. The mean number of spontaneous turns (control) recorded in the absence of any drug was 5.8 ± 1.3. The results with 1 mg/kg quipazine and 1 mg/kg 5-MDT were significantly greater than control ($p<0.05$; Mann-Whitney U test).

Statistical evaluation of the circling was performed using the non-parametric Mann-Whitney U test.

At the completion of the experiments, a sample of the rats with medial raphe lesions were anaesthetized with pentobarbitone sodium. The brains were fixed by intracardiac perfusion with heparinized saline followed by buffered formal-saline. After post-fixation (1 week), serial 15 μm sections were cut and stained with luxol fast blue-basic fuchsin. The raphe lesions were confirmed histologically. The remaining lesioned rats were sacrificed by decapitation, the brains were removed and the striata dissected out. The 5-HT and dopamine in individual striata was extracted and assayed fluorimetrically [11].

The drugs used were: d-amphetamine sulphate (British Drug Houses), apomorphine hydrochloride (MacFarlan Smith), 5-methoxy-N,N-dimethyltryptamine (Sigma), fenfluramine hydrochloride and quipazine were gifts from Servier laboratories and Miles Laboratories.

RESULTS

Immediately after surgery, rats with asymmetric medial raphe lesions turned in tight circles towards the non-lesioned side. After 2 weeks the circling had diminished greatly, and saline-treated, lesioned rats made wide circles during their normal exploratory behaviour. The mean number of complete turns varied from 4.6–6.6 turns/10 min (see legends to Figs. 1–3). The 5-HT receptor agonists quipazine (1 mg/kg IP) and 5-methoxy-N,N-dimethyltryptamine (5-MDT; 1 mg/kg IP) caused statistically significant increases in the contraversive circling rates (Fig. 1). However, larger doses of both 5-HT agonists had no significant effect on the mean circling rates. This can be attributed in part to the increasing incidence of motor effects including head-twitches, forepaw treading and lateral extension of the limbs. The nature of the circling produced by the lowest doses of quipazine and 5-MDT was noticeably different from the spontaneous circling in that the rats turned in tighter circles and ceased to explore the open field area. Fenfluramine (1–10 mg/kg IP), a compound that releases endogenous 5-HT, had no effect on the mean circling rate (Fig. 1).

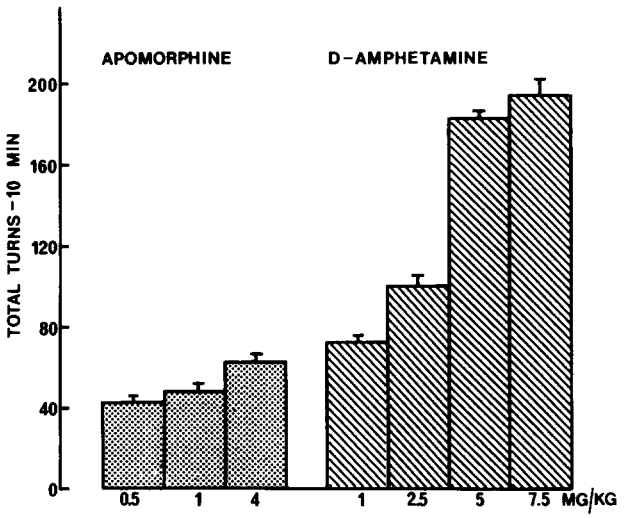

FIG. 2. The contralateral circling produced by apomorphine (0.5–4 mg/kg IP, 20–30 min) and d-amphetamine (1–7.5 mg/kg IP, 30–40 min) in rats with an asymmetric lesion of the medial raphe nucleus. Each result is the mean obtained with 8 rats. Vertical bars represent SEM. The mean number of spontaneous turns (control) recorded in the absence of any drug was 4.6 ± 2.1. All the results shown in the figure are significantly greater than control ($p<0.01$; Mann-Whitney U test).

Both apomorphine (0.5–4 mg/kg), a direct dopamine receptor agonist [1], and d-amphetamine (1–7.5 mg/kg), a compound that releases endogenous dopamine [3], caused dose-related increases in contralateral circling rates in raphe lesioned rats (Fig. 2). The maximum circling rates were re-

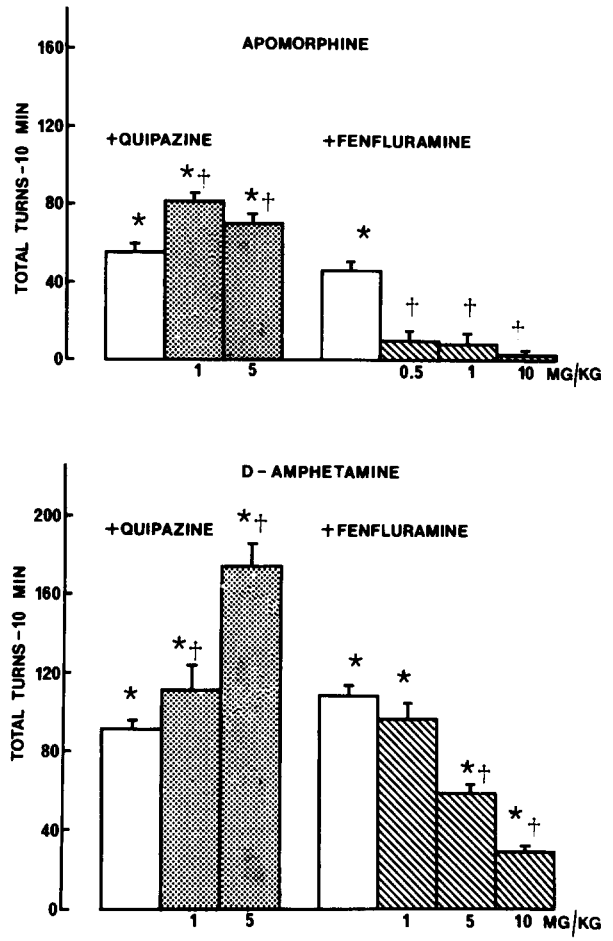

FIG. 3. The contralateral circling produced by apomorphine (upper figure) and d-amphetamine (lower figure) in asymmetric medial raphe lesioned rats pretreated with quipazine (1 and 5 mg/kg) and fenfluramine (1–10 mg/kg). Open bars show effect of apomorphine (1 mg/kg, 20–30 min) and d-amphetamine (2.5 mg/kg, 30–40 min) alone. Dotted/shaded bars show effects of quipazine/fenfluramine pretreatment on the apomorphine and d-amphetamine circling. Quipazine was administered 5 min before and fenfluramine 15 min before apomorphine/d-amphetamine. Each result is the mean obtained with 8 rats. Vertical bars represent SEM. The mean number of spontaneous turns (control) recorded in the absence of any drug was 6.6 ± 1.9 (upper figure) and 4.3 ± 2.1 (lower figure). *Significantly greater than spontaneous turns ($p<0.01$, Mann Whitney U test). †Significantly different from apomorphine/d-amphetamine alone ($p<0.01$).

corded 20–30 min after apomorphine and 30–40 min after d-amphetamine. Several doses of the dopamine agonists were administered over a 10 day period to groups of raphe lesioned rats. The mean circling rates remained constant.

The effects of 5-HT agonists on the dopamine induced circling were investigated using standard, submaximal doses of apomorphine (1 mg/kg) and d-amphetamine (2.5 mg/kg). Groups of asymmetric raphe lesioned rats were pretreated with different doses of quipazine and fenfluramine and the effect on the circling rates of rats treated with apomorphine and d-amphetamine was determined. Apomorphine increased the rate of circling. Quipazine (1.5 mg/kg) given 5 min before apomorphine and d-amphetamine produced a further increase in the circling (Fig. 3). In contrast, fenfluramine, administered 15 min before apomorphine or d-amphetamine, antagonized the circling produced by both dopamine agonists. The circling rate of rats treated with apomorphine and fenfluramine was not significantly different from the normal, spontaneous circling. The results presented in Fig. 3 show that quipazine and fenfluramine produced changes in the d-amphetamine circling rates that were closely dose-related.

The amount of 5-HT and dopamine measured in striata removed from normal and raphe lesioned rats is shown in Table 1. Asymmetric raphe lesions, the size and location of which are represented in Fig. 4, caused a statistically significant reduction in the 5-HT content of the ipsilateral striatum together with a small increase in the contralateral striatal 5-HT level. There were no changes in striatal dopamine content.

DISCUSSION

The aim of the present study was to find a 5-HT rotation model analogous to the dopamine circling model. The dopamine model has a striatal imbalance produced by selective lesioning of the nigro-striatal dopamine neurones with 6-hydroxydopamine. The lesion, together with the development of dopamine receptor supersensitivity, explains why apomorphine causes circling in one direction but d-amphetamine, by releasing more dopamine in the intact striatum, causes circling in the opposite direction [8]. It is a straightforward procedure to lesion unilaterally the 5-HT projection from the medial raphe to the striatum to produce a specific 5-HT lesion [7,14]. Equally, 5-HT receptors develop denervation supersensitivity, although they are possibly more resistant to change than dopamine receptors because, unlike dopamine receptors, they are not affected by short-term pharmacological depletion of 5-HT [11, 13, 19, 23]. Fi-

TABLE 1

EFFECT OF LEFT MEDIAL RAPHE LESIONS ON THE 5-HYDROXYTRYPTAMINE (5-HT) AND DOPAMINE (DA) CONTENT OF RAT STRIATUM

Lesion	Left striatum		Right striatum	
	5-HT* (μg/g)	DA*	5-HT* (μg/g)	DA*
None	1.39 ± 0.01	6.43 ± 0.21	1.27 ± 0.02	6.67 ± 0.33
Left medial raphe	0.60 ± 0.10†	6.29 ± 0.20	1.40 ± 0.02†	6.35 ± 0.09

*Data expressed as mean of 8 ± SEM. Significance of difference between lesioned and non-lesioned results: †$p<0.01$ (Student's t-test).

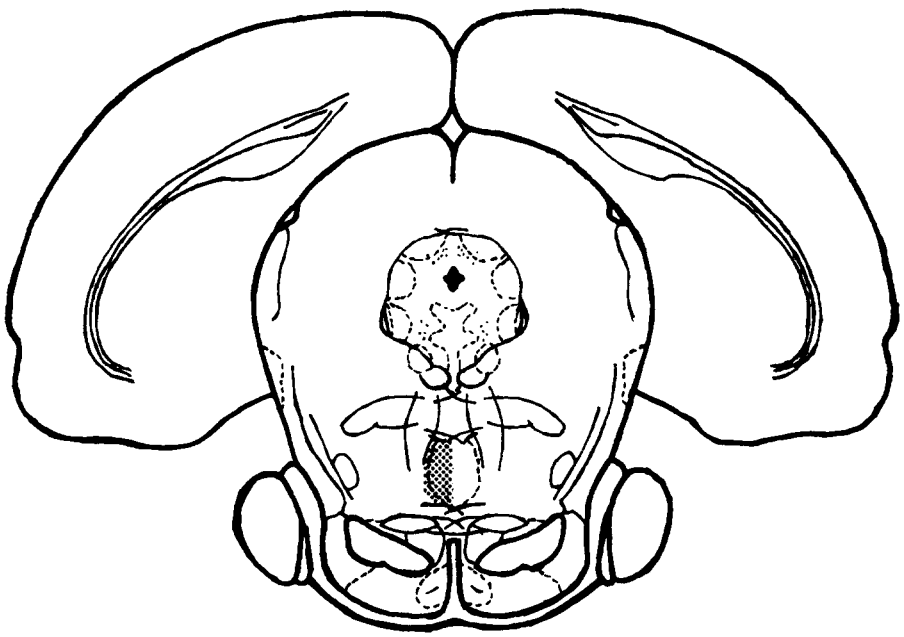

FIG. 4. Diagrammatic representation of the localisation and extent of damage caused by an electrolytic, asymmetric lesion of the medial raphe nucleus (hatched area) in the rat. Anterior plane is 0.3 mm.

nally, there are specific 5-HT receptor agonists, such as quipazine and 5-MDT [9,16] as well as compounds such as fenfluramine that rapidly release endogenous 5-HT [5,20]. Thus, in theory, it should be possible to have a rotation model in which quipazine causes rotation in one direction while fenfluramine causes rotation in the opposite direction.

A previous attempt to make a 5-HT rotation model used the neurotoxic agent 5,7-dihydroxytryptamine to lesion the medial forebrain bundle of the rats [10]. The 5-HT precursor 5-hydroxytryptamine increased the resulting spontaneous contralateral circling whereas p-chloroamphetamine, which is thought to release 5-HT, caused ipsilateral turning. However, there are a number of problems inherent in this method. One is that neurotoxic agents do not always produce specific 5-HT lesions. Not only is there a non-specific, vehicle-induced effect [22], but there is occasionally loss of forebrain dopamine [18], although this problem does not arise with 5,7-dihydroxytryptamine [10]. Secondly, p-chloroamphetamine not only releases 5-HT but also noradrenaline [15]. Finally, 5-HT agonists cause such extremely slow circling that it is very difficult to distinguish between spontaneous and drug-induced rotation.

In the present study, the aim was to produce a specific 5-HT lesion by lesioning the midbrain raphe nucleus. The medial raphe was chosen as the site for the electrolytic lesion because it contains a proportion of the 5-HT neurones that project to the neostriatum [14]. Although quipazine and 5-MDT increased the contralateral circling rate, neither appeared to have a dose-related effect. More importantly, fenfluramine did not produce any ipsilateral circling in the present experiments and was very weak in causing ipsilateral rotation in rats with 5,7-dihydroxytryptamine lesion of the medial forebrain bundle [22]. It is concluded that, probably because of the nature of the 5-HT innervation of the striatum, it is not possible to have a 5-HT rotation model exactly analogous to the dopamine model.

The dopamine agonists apomorphine and d-amphetamine, in agreement with earlier reports [6,7], caused vigorous contralateral circling in rats with a medial raphe lesion. d-Amphetamine had the most dose-related effect, possibly because the loss of striatal 5-HT has a greater effect on dopamine release mechanisms than on dopamine receptor mediated events. This conclusion is consistent with the idea that 5-HT normally modulates striatal dopamine release [14]. For this reason, it was decided to explore the possibility that a 5-HT-dopamine release interaction might provide a better model for 5-HT function than the interaction between 5-HT drugs and apomorphine. The results demonstrate that the 5-HT agonist quipazine and the 5-HT releaser fenfluramine have potent, dose-related effects on d-amphetamine circling in raphe lesioned rats. Equally significant is the fact that the two types of 5-HT drugs have effects which are opposite to each other.

The role of striatal 5-HT in relation to other neurotransmitters, especially dopamine and acetylcholine, is not clear. One possibility is that 5-HT tonically inhibits striatal dopamine function [14] while another suggestion favours a positive cooperation between 5-HT and dopamine [22]. One difficultly in deciding on the exact relationship is that 5-HT neurones, especially those from the dorsal raphe nucleus, project to the substantia nigra and influence the function of the nigro-striatal dopamine system [27]. The suggestion that 5-HT inhibits striatal dopamine function could explain why dopamine agonists cause circling in raphe lesioned rats since the loss of 5-HT should disinhibit striatal dopamine release. However, a 5-HT agonist such as quipazine should reduce dopamine release and antagonize dopamine induced circling in raphe lesioned rats. In fact, as the present results show, the reverse is true and quipazine potentiated both apomorphine and d-amphetamine circling. These findings support the idea that 5-HT and dopamine function cooperatively. Thus quipazine increases d-amphetamine circling by enhancing dopamine release while fenfluramine, by acting predominantly in the intact striatum to release 5-HT and increase

dopamine function, would reduce the imbalance between the two sides of the brain and antagonize d-amphetamine circling.

In conclusion, modulation of d-amphetamine circling by 5-HT agonists in rats with a specific 5-HT lesion may provide the basis for a rat circling model which might prove valuable for studying some aspects of brain 5-HT function.

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