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Rotational behaviour elicited by 5-HT in the rat: evidence for an inhibitory role of 5-HT in the substantia nigra and corpus striatum

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Unilateral intranigral microinjection of 5-HT or Wy 25093, a selective inhibitor of 5-HT uptake, elicited ipsiversive circling and a fall in striatal dopamine (DA) turnover. Conversely intranigral methysergide or unilateral lesions of the raphé-nigral 5-HT pathway produced contraversive turning and/or increased DA turnover. Both types of behaviour were accentuated by nialamide and attenuated by haloperidol. Caudate injections of 5-HT or Wy 25093 provoked contraversive turning which was increased by nialamide and hyoscine, partially diminished by haloperidol and antagonized by eserine. Ipsiversive circling was induced by intracaudate methysergide, which was potentiated by nialamide and eserine, unaffected by haloperidol and depressed by hyoscine. It is proposed that 5-HT normally subserves an inhibitory function both in the substantia nigra and corpus striatum.

The substantia nigra (SN) and corpus striatum (CS) both receive 5-hydroxytryptaminergic inputs from the midbrain raphé nuclei (for detailed refs see Waldmeier & Delini-Stula 1979). The precise role of 5-hydroxytryptamine (5-HT) in these terminal regions has not yet been firmly established, although preliminary findings indicate that 5-HT may influence the normal functioning of the nigrostriatal dopamine (DA) system in a complex fashion (for reviews see Kostowski 1975; Samanin & Garattini 1975). For example, electrical stimulation of the dorsal raphé nuclei was observed to produce a methysergide-sensitive depression of single unit activity in the caudate-putamen, consistent with 5-HT having an inhibitory function there(Miller et al 1975; Olpe & Koella 1977). On the other hand, administration of 5-HT agonists and uptake inhibitors to rats has been reported to potentiate both the catalepsy (Carter & Pycock 1977) and the increase in striatal DA turnover (Waldmeier & Delini-Stula 1979) elicited by haloperidol. These results suggested to the latter authors there was an excitatory 5-HTergic input to striatal cholinergic interneurons located within the striatonigral feedback loop. However, this interpretation would be incorrect if the primary site of action of 5-HT (Besson et al 1969) and haloperidol (Tulloch et al 1978) for releasing DA in these experiments was on the membrane of the DA-containing nerve terminals (i.e. presynaptic).

Little is known of the function of 5-HT in the SN, except that nigral neurons are predominantly inhibited by iontophoretic 5-HT, whereas lesions of the raphé-nigral 5-HT pathway and inhibitors of 5-HT synthesis cause apparent DAergic hyperactivity (Dray et al 1978; Tanner 1978). The aim of the present study, therefore, was to investigate the effects of modifying 5-HT receptor activity in the SN and CS of one hemisphere on nigrostriatal DA cell activity, as judged by the development of rotational behaviour coupled with an altered DA utilization. Thus contraversive circling accompanied by a decrease in DA content and/or an increase in homovanillic acid (HVA) concentration in the ipsilateral striatum (i.e. an increased DA turnover—see Korf et al 1976) was taken to represent an activation of the nigrostriatal DA pathway, and vice versa. Our results support the concept of an inhibitory 5-HT-DA link in the SN and an inhibitory synapse between 5-HT and acetylcholine-containing neurons in the CS.

METHODS AND MATERIALS

Male Wistar albino rats (160–180 g) were lightly anaesthetized with halothane and drug solutions or vehicle injected stereotaxically into the SN, CS or other specified brain regions using coordinates derived from König & Klippel (1963). Injections into the SN were usually restricted to volumes of 0·1–0·2 μl and expelled slowly over 5 min. Upon recovery from anaesthesia (about 5 min) animals were placed in an open field and observed for signs of bilateral postural or locomotor asymmetry. The direction and frequency of tight circling on the spot, as distinct from random exploratory movements,

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was noted and scored. Behaviour profiles were compared by analysis of variance. Separate groups of animals were similarly treated and killed at the height of turning for fluorimetric analysis of striatal DA (Shellenberger & Gordon 1971) and HVA contents (Murphy et al 1969) using established methods. In another group of animals the raphé-nigral 5-HT pathway was partially destroyed by injecting 5,6-dihydroxytryptamine (DHT, 20 μg in 2 μl) into one SN. Fifteen days later the noradrenaline (NA, Shellenberger & Gordon 1971) and 5-HT concentrations (Curzon & Green 1970) were measured in lesioned and intact nigras by conventional fluorimetric analysis.

In all experiments HVA and monoamine concentrations were tested for bilateral differences by paired *t*-test, whilst unpaired *t*-test was used to compare treated animals with controls.

Uptake studies were performed using 0·15 mm cubes of freshly dissected SN tissue as described in detail elsewhere (Starr 1978). Briefly, suspensions of SN tissue in Krebs bicarbonate solution were preincubated for 10 min at 37 °C, with or without added drug, before measuring the accumulation of radioactive substrate (10^{-7} M) over 10 min by liquid scintillation spectrometry. Uptake was expressed as the tissue : medium (T/M) radio (d min^{-1} g^{-1} wet wt tissue : d min^{-1} ml^{-1} medium). Results were analysed by *t*-test.

(+)-Amphetamine sulphate, eserine sulphate and hyoscine hydrobromide were from Sigma, apomorphine hydrochloride from Macfarlan Smith and haloperidol from Searle. Desmethylimipramine was given by Dr. M. J. Neal, while generous gifts of Wy 25093 (1-[1-([indol-3-yl]methyl)piperid-4-yl]-3-benzoylurea, hydrochloride) from Wyeth and nialamide from Pfizer are also gratefully acknowledged.

RESULTS

Nigral injections

Unilateral intranigral injection of 5-HT (10 μg in 0·1 μl 0·9% NaCl (saline), invariably produced slow, tight, ipsiversive circling on the spot, which began immediately upon recovery from anaesthesia, reached a peak frequency at 20 min and lasted for 40 min (Fig. 1a). Varying the injection coordinates rostrocaudally (A 1·8–2·4), dorsoventrally (D −2·5–3·0) or mediolaterally (L 1·75–2·5) according to the stereotaxic atlas of König & Klippel (1963), failed to modify the type or intensity of turning behaviour produced. This treatment lowered striatal DA turnover ipsilaterally; at 20 min post-injection DA levels were increased by 14·9% (P <0·01) and homovanillic acid (HVA) levels reduced by 33·3% (P <0·01, n = 6, Table 1) when compared with those of the untreated hemisphere, or with those of control rats injected with saline (not shown). No turning occurred when 5-HT was applied to neurons in the mesencephalic reticular formation (A 2·8, D −2·0, L 2·2), crus cerebri (A 3·4, D −3·0, L 2·1) or medial lemniscus (A 2·6, D −1·6, L 2·0), or when physiological saline was injected into the SN of control animals.

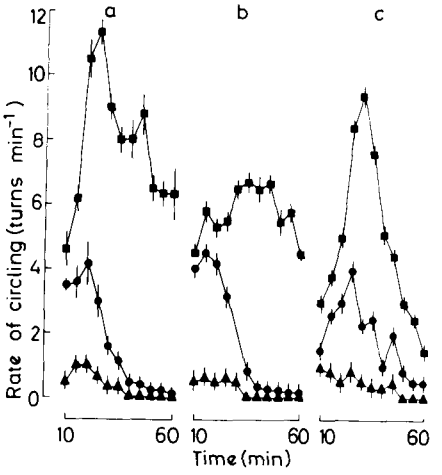

FIG. 1. Rate of circling (turns min^{-1}) (ordinate) at different times (min) (abscissa) after unilateral intranigral injection of 10 μg 5-HT (a) (ipsiversive), 10 μg Wy 25093 (b) (ipsiversive) and 1 μg methysergide (c) (contraversive) in saline-treated control rats (circles) and rats pretreated i.p. with nialamide (100 mg kg^{-1} 2 h earlier) (squares) or haloperidol (0·5 mg kg^{-1} 15 min earlier) (triangles). Each point is the mean ± s.e.m. of 10 determinations.

Pretreating animals with the monoamine oxidase inhibitor nialamide (100 mg kg^{-1} i.p. 2 h earlier) markedly increased the maximum rate of circling evoked by intranigral 5-HT (+172%, P <0·001; Fig. 1a), and greatly prolonged its duration. Conversely the DA receptor blocker haloperidol (0·5

Table 1. Effects of 5-HT (10 μg, 20 min), Wy 25093 (10 μg, 20 min), methysergide (1 μg, 20 min) and DHT (20 μg, 15 days) injected into one nigra on striatal DA (μg g^{-1}) and HVA (μg g^{-1}) concentrations.

	DA		HVA	
Treatment	Injected side	Control side	Injected side	Control side
5-HT	3·48 ± 0·21*	3·03 ± 0·18	0·26 ± 0·15*	0·39 ± 0·02
Wy 25093	3·39 ± 0·17*	3·11 ± 0·16	0·27 ± 0·02*	0·37 ± 0·03
Methysergide	2·96 ± 0·12*	3·25 ± 0·13	0·54 ± 0·04**	0·38 ± 0·02
DHT	2·60 ± 0·14*	3·28 ± 0·20	0·57 ± 0·04**	0·35 ± 0·02

Each value is the mean ± s.e.m. of six determinations.
* P < 0·01 ** P < 0·001 versus controls by paired *t*-test.

mg kg^{-1} i.p. 15 min beforehand) severely curtailed this circling response (−76%, P <0·001; Fig. 1a).

The 5-HT uptake blocker Wy 25093 was also tested for its ability to elicit locomotor asymmetry by potentiating the postsynaptyic actions of the endogenously released amine. Fig. 1b shows that 10 μg of this drug (0·2 μl) given into one SN elicited ipsiversive turning behaviour which was indistinguishable in intensity and time course from that evoked by 5-HT, and which was likewise accentuated by nialamide and attenuated by haloperidol (P <0·001). At the peak of this effect (20 min) the level of DA in the corresponding striatum was slightly increased (+9·0%, P <0·01) and HVA reduced (−27·1%, P <0·01, Table 1) compared with the opposite CS, indicating DA turnover at this time was lower on the injected side of the brain.

The specificity of Wy 25093 as an inhibitor of 5-HT transport into SN tissue was also determined. In 4 experiments mean 5-HT uptake was reduced from a T/M ratio of 36·6 ± 5·4 in controls to 6·7 ± 0·6 (P <0·001) in the presence of 10^{-4} M Wy 25093. The corresponding uptakes of NA (T/M ratio = 8·6 ± 1·0) and DA (T/M ratio = 11·2 ± 0·9) were not significantly altered by this concentration of Wy 25093.

Blocking nigral 5-HT receptors unilaterally by injecting methysergide (1 μg in 0·1 μl) initiated contraversive circling which was significantly increased by nialamide (+137%, P <0·001) and reduced by haloperidol (−80%, P <0·001, Fig. 1c). Twenty min after methysergide administration, striatal DA turnover had risen on the treated side, as evidenced by the slight fall in DA (−9%, P <0·01) and the substantial rise in HVA (+42%, P <0·001, Table 1).

In a further set of experiments the 5-HTergic input to one SN was interrupted by injecting 5,6-dihydroxytryptamine (DHT, 20 μg in 2 μl) intranigrally. To prevent the neurotoxin being taken up into and subsequently destroying NA-containing nerve terminals, the rats were first pretreated i.p. with 500 mg kg^{-1} desmethylimipramine. Fifteen days later the 5-HT contents of lesioned nigras were only 0·67 ± 0·03 μg g^{-1} compared with 5·52 ± 0·17 μg g^{-1} in controls (P <0·001), whereas nigral NA concentrations were reduced, though not significantly, from 0·86 ± 0·03 to 0·78 ± 0·03 μg g^{-1}. Rats thus lesioned did not circle spontaneously, but were stimulated to turn towards the lesioned side when challenged i.p. with either amphetamine (2·5 mg kg^{-1}) or apomorphine (0·5 mg kg^{-1}). Neither drug was effective in this respect in sham-lesioned rats that had received 2 μl of the toxin vehicle intranigrally (i.e. physiological saline containing 0·1% ascorbic acid). Biochemical analysis of the CS revealed a consistent increase in DA turnover on the same side as the DHT lesion; fifteen days after the DHT injection the ipsilateral striatal DA concentration had fallen by 21% (P <0·01) and the HVA concentration had risen by 63% (P <0·001) compared with controls (Table 1).

Caudate injections

Much larger doses of the above drugs were required to initiate any form of turning behaviour from the CS (coordinates A 6·7–7·5, D +1·0–(−)1·0 and L 2·5–4·0; König & Klippel 1963). Ten μg of 5-HT (2 μl) given into one CS caused the animals to adopt an asymmetric posture with the upper trunk and head twisted towards the uninjected side, which developed into weak contraversive circling at a ten-fold higher dose (Fig. 2a). This turning motion was markedly facilitated by pretreatment with either nialamide (+111%, P <0·001, Fig. 2a) or hyoscine (+189%, P <0·001, 2·5 mg kg^{-1} i.p. 15 min earlier), though neither potentiation was attenuated by haloperidol (not shown). The results of administering haloperidol to rats receiving 5-HT alone

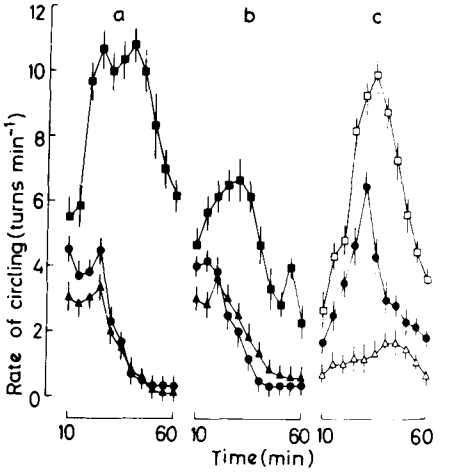

FIG. 2. Rate of circling (turns min^{-1}) (ordinate) at different times (min) (abscissa) after unilateral intracaudate injection of 100 μg 5-HT (a) (contraversive), 200 μg Wy 25093 (b) (contraversive) and 10 μg methysergide (c) (ipsiversive) in saline-treated control rats (circles) and rats pretreated i.p. with nialamide (100 mg kg^{-1} 2 h earlier) (solid squares), haloperidol (0·5 mg kg^{-1} 15 min earlier) (solid triangles), eserine (2 mg kg^{-1} 30 min earlier) (open squares) or hyoscine (2·5 mg kg^{-1} 15 min earlier) (open triangles). Each point is the mean ± s.e.m. of 10 determinations.

were less clear cut. It would appear from Fig. 2a there was a minor, early component to the 5-HT turning response which was susceptible to inhibition by haloperidol. On the other hand, eserine (2 mg kg^{-1} i.p. 30 min earlier) antagonized the 5-HT evoked rotations (-26%, $P < 0{\cdot}005$). Similar results were obtained with 200 μg intracaudate Wy 25093 (see Fig. 2b). Methysergide (10 μg in 2 μl) had the opposite effect; rats turned slowly in tight ipsiversive circles with a mean maximum frequency of $6{\cdot}5 \pm 0{\cdot}4$ turns min^{-1} after 30 min. This behaviour was unaltered by haloperidol (not shown), but was clearly exaggerated by eserine and antagonized by hyoscine (doses as before). These results are illustrated in Fig. 2c.

DISCUSSION

One way of introducing bilateral asymmetry into an animal's posture and movements is to create an imbalance in the level of DA receptor stimulation between the two striata (Ungerstedt 1971). The animal will then turn towards the lesser activated side. It is plausible to assume, therefore, that intranigral 5-HT causes ipsiversive rotation and a drop in striatal DA turnover by diminishing DAergic activity on the injected side of the brain. The similar efficacy of a 5-HT uptake blocker (Wy 25093) and the opposite action of a 5-HT receptor antagonist (methysergide), presumably by facilitating and inhibiting synaptically released 5-HT respectively, lend weight to this interpretation. These results also suggest that the raphé-nigral 5-HT pathway is tonically active and exercises a continuous inhibitory restraint over the nigrostriatal DA system. According to this hypothesis one would predict that chemical denervation of the 5-HT afferents to the SN would effectively disinhibit (i.e. excite) the DA neurons and thereby elevate the turnover rate of their transmitter in the corresponding CS. This is precisely what we observed in the unilaterally DHT-lesioned rats and further testifies to the correctness of our model. Dray et al (1977) speculated that in such circumstances elevated DA release may eventually lead to compensatory hyposensitivity of the postsynaptic DA receptors in the CS. This is an attractive possibility and could explain why both amphetamine and apomorphine always stimulated our lesioned rats to turn towards the denervated (i.e. less sensitive) striatum.

Since none of the drugs employed in the present experiments was able to modify the animal's behaviour when applied to neurons adjacent to the SN, we conclude that the 5-HT receptors mediating the behavioural responses described here are specifically localized to this nucleus. Bearing in mind that iontophoretically administered 5-HT predominantly suppresses single unit firing in the SN (e.g. Dray et al 1978), the simplest neuronal circuitry which accommodates these experimental data is where the axon terminals of the raphé-nigral 5-HTergic fibres synapse directly with the nigrostriatal DA neurons at inhibitory junctions.

Modification of these turning behaviours by nialamide and haloperidol is thought to be directed principally at the DA released in the CS and therefore secondary to any drug-induced changes in 5-HT activity within the SN. As observed above nialamide will tend to exaggerate any bilateral asymmetry by protecting striatal DA (and presumably injected or endogenously released 5-HT as well) against oxidative deamination, whereas haloperidol, by blocking the postsynaptic actions of released DA, would be expected to neutralize any imbalance in DAergic activity between the two hemispheres. Moreover administering nialamide and haloperidol by the i.p. route ensures that these drugs reach both halves of the brain and explains why drug-induced turning in either direction may be equally susceptible to their actions.

The CS is among the forebrain regions innervated by the raphé nuclei (Fuxe 1965). In agreement with Costall et al (1976) it proved to be much more difficult to initiate rotational behaviour by injecting drugs directly into the CS, even when large drug doses and injection volumes were used. Perhaps this reflects, for example, the barriers to diffusion encountered by the drug molecules and/or the rapidity with which these compounds are bound or inactivated by striatal tissue. Nevertheless, the weak contraversive circling elicited by intracaudate 5-HT and Wy 25093, together with the contrasting effect of methysergide, is compatible with 5-HT having a tonic inhibitory function there. Since DA receptor blockade only weakly and partially antagonized these behaviours, it seems likely that 5-HT acts predominantly via receptors located postsynaptically on caudate neurons. However the small haloperidol-sensitive component of the turning movements evoked by striatal 5-HT should not be ignored, because it implies the indoleamine may also act indirectly by liberating DA from the terminals of nigrostriatal DA neurons (see Besson et al 1969). Any such contribution to the behavioural responses must necessarily be small, as the nialamide-potentiated circling was not modified by haloperidol. By implication, therefore, the enhancement

of the responses by nialamide in this instance is most probably due to slower enzymic breakdown of the injected (or released) 5-HT.

The facilitation by hyoscine and the attenuation by eserine of the 5-HT induced contraversive circling, together with the converse effects of these drugs on methysergide-evoked ipsiversive rotations, are consistent with raphé-caudate 5-HT fibres (like those of the nigrostriatal DA system) synapsing directly with, and inhibiting, striatal cholinergic neurons. Although we did not measure acetylcholine turnover in this study, we predict it should be raised by blocking postsynaptic 5-HT receptors in the caudate nucleus. Our proposal need not conflict with the recent observation by Waldmeier & Delini-Stula (1979) that drugs which facilitate 5-HT neurotransmission in the CS also exaggerate the pharmacological effects of haloperidol, as their postulated excitatory role for 5-HT in this region may be true of its DA-releasing property at presynaptic sites (Besson et al 1969; Tulloch et al 1978). In either event the net outcome of raising striatal 5-HT concentrations would be to increase the synaptic concentration of inhibitory transmitter (5-HT and/or DA), which would suppress cholinergic cell firing and initiate contraversive turning.

The overall conclusion from these experiments is that 5-HT is able to depress the electrical activity both of the nigrostriatal DA neurons and of those cells in the CS controlled by these DAergic fibres. Our interpretation could provide an explanation for the apparently anomalous situation recently described by Milson & Pycock (1976). These authors discovered that circling behaviour mediated by striatal DA receptor activation was diminished by raising brain 5-HT concentrations with systemically administered 5-hydroxytryptophan (5-HT). By contrast, clomipramine, a drug that potently inhibits neuronal 5-HT uptake and which would likewise be predicted to facilitate 5-HT neurotransmission in the c.n.s., accelerated the rate of turning. This paradox would be resolved if 5-HTP stimulated 5-HT biosynthesis more effectively in the SN and clomipramine inhibited 5-HT transport principally in the CS. According to this model the raphé-caudate 5-HT fibres strengthen, whereas the raphé-nigral neurons oppose the influence of the nigrostriatal DA system on locomotor behaviour. That is, the two 5-HT inputs to the basal ganglia act in opposition. Further experiments are required to clarify this point and to determine how the balance between these two 5-HT pathways is maintained and to what extent they contribute to the normal control of DA-mediated behaviours.

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