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Evidence that 5-HT agonist-induced rotational behaviour in the rat is mediated via 5-HT_1 receptors

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Abstract. The rotational behaviour induced by 5-HT agonists has been investigated in rats with lesions of the dorsal raphe' nucleus (DRN). We have previously reported that 5-methyoxy-N,N-dimethyl-tryptamine (5-MeODMT) caused dose-related contralateral rotation in rats with 5,7-dihydroxytryptamine (5,7-DHT) lesions of the DRN. Similar findings are now presented for the 5-HT_1 agonists 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and 5-methoxy-3 (1,2,3,6-tetrahydropyridin-4-yl) (1H indole) (RU24969). In this model, in agreement with the behavioural studies, both agonists were shown to have a greater affinity for the 5-HT_1 binding site when compared with the 5-HT_2 binding site. Antagonist studies using selective 5-HT_2 antagonists (ketanserin and pirenperone) at non-sedative doses failed to inhibit this behaviour. In contrast, the classical 5-HT antagonist methysergide caused significant inhibition of the rotational behaviour. These results suggest that 5-HT agonist-induced rotation in the rat is mediated via 5-HT_1 receptors, probably located in the substantia nigra.

Key words: Supersensitivity – 5-HT_1 receptors – Methysergide – Ketanserin – Pirenperone – 5-HT agonist rotational behaviour – Rat

Recently, radioligand binding studies in vitro have suggested that 5-HT receptors can be divided into two subtypes, 5-HT_1 and 5-HT_2 (Peroutka and Snyder 1979; Leysen et al. 1981). These workers have also indicated that behavioural changes induced by either 5-HT itself or by 5-HT agonists are generally associated with the 5-HT_2 site. As yet, no specific behavioural study has been described where the behaviour is associated with an action at 5-HT_1 receptors. We have previously demonstrated rotational behaviour after 5-HT agonist injection in rats with a unilateral 5,7-dihydroxytryptamine (5,7-DHT) lesion of the dorsal raphe' nucleus (DRN) (Blackburn et al. 1980). We now present data on the effects of several 5-HT agonists in this model, in particular, the recently described 5-HT_1 agonists 8-hydroxy-2-(di-*n*-propyl-amino)tetralin (8-OH-DPAT) (Arvidsson et al. 1981) and 5-methyoxy-3(1,2,3,6-tetrahydropyridin-4-yl) (1H-indole) (RU24969) (Hunt et al. 1981) in an atempt to characterise the 5-HT receptors involved in this response.

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Materials and methods

Lesions. The method used was that described previously (Blackburn et al. 1980). Adult male rats of the Alderley Park strain (180–220 g) were anaesthetised with halothane (3% v/v O_2) and placed in a David Kopf stereotoxic instrument. Unilateral lesions of the DRN (co-ordinates: A = 0.2, H = 5.0, L = 0.3 mm from dura, according to Konig and Klippel 1963) were induced with 5,7-DHT (16 µg salt/2 µl 0.2% ascorbic acid solution) injected 1 h after an IP injection of pargyline 50 mg/kg (Sigma).

After completion of an experiment, histological verification of the lesion site and biochemical estimations of the selectivity of neurotoxin were carried out as previously described (Blackburn et al. 1980).

Rotation. On the 4th day after lesioning of the DRN all rats were assessed for turning behaviour by challenging with 5-methoxydimethyltryptamine (5-MeODMT 7.5 mg/kg SC) and assessed in automated rotometers for a period of 2 h. The lesion was considered successful when a rat made at least 200 turns within the 2 h assessment period. Drug-induced turning behaviour was then assessed with at least 72 h intervals between successive drug injections (SC). Control rats were injected with appropriate vehicle and always run simultaneously. Rotation was measured after injection of the following drugs: 5-methoxy-N,N-dimethyltryptamine (5 MeODMT) 1–10 mg/kg SC (Sigma); Quipazine 10–30 mg/kg SC (Dr. R. J. Pearce, ICI); 6-chloro-2-(1-piperazinyl)pyrazine (MK212) 2.5–5.0 mg/kg SC (M.S.D.); 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OHDPAT) 0.1–7.5 mg/kg SC (Dr. M. T. Cox, ICI); and 5-methoxy-3-(1,2,3,6-tetrahydropyradin-4-yl) 1H-indole (RU24969) 0.1–7.5 mg/kg SC (Dr. D. LeCount, ICI). All drugs were dissolved in 0.9% saline, with the exception of ketanserin and pirenperone which were dissolved initially in 0.1% lactic acid. Antagonists used were methysergide (Sandoz Pharmaceuticals) ketanserin and pirenperone (Janssen Pharmaceutical, Beerse, Belgium).

Biochemical studies. The measurement of [^{3}H]-5-HT and [^{3}H]-spiroperidol binding in cortical tissue, was performed essentially by the method described by Peroutka and Snyder (1979).

Results

Rotation. The results presented in Table 1 show that 5-MeODMT, 8-OHDPAT and RU24969 caused a dose-re-

Table 1. Effect of 5-HT agonists in DRN-lesioned rats

Agonist	Dose mg/kg SC	Turning behaviour	
		Net mean no. of contralateral turns ± SE[a]	Net mean no. of ipsilateral turns ± SE[a]
Saline	0.2 ml	0.17 ± 11 (12)	0.1 ± 6 (12)
5-MeODMT	1.0	89 ± 12 (8)	
	2.5	122 ± 25 (9)	
	7.5	213 ± 15 (13)	
	10.0	257 ± 17 (24)	
8-OH-DPAT	0.1	87 ± 12 (6)	
	0.5	264 ± 54 (9)	
	2.0	380 ± 44 (17)	
	3.75	319 ± 62 (8)	
	7.5	285 ± 29 (8)	
RU24969	0.1	29.7 ± 11 (9)	
	0.5	216 ± 18 (11)	
	0.75	238 ± 41 (6)	
	1.0	287 ± 43 (6)	
	3.75	425 ± 58 (5)	
	7.5	342 ± 41 (5)	
Quipazine	10.0		28 ± 29 (4)
	30.0	7 ± 27 (4)	
MK212	2.5		14 ± 6 (4)
	5.0	3 ± 4 (4)	

Figures in parentheses indicate number of animals tested
[a] In 2 h

Table 2. Effect of antagonists on 5-HT agonist induced turning behaviour in DRN-lesioned rats

Drugs	Dose mg/kg SC	Turning behaviour	
		Mean (± SE) per 2 h	No. of turns
		Treated	Control
Ketanserin	0.5	130 ± 7	160 ± 26 (5)[a]
+ 5-MeODMT	7.5		
Pirenperone	0.05	202 ± 28	160 ± 37 (7)
+ 5-MeODMT	7.5		
Methysergide	10.0	129 ± 33*	236 ± 50 (8)
+ 5-MeODMT	7.5		
Ketanserin	0.5	353 ± 93	365 ± 65 (6)
+ 8-OH-DPAT	2.0		
Pirenperone	0.05	340 ± 40	404 ± 42 (7)
+ 8-OH DPAT	2.0		
Ketanserin	0.5	345 ± 63	375 ± 53 (8)
+ RU24696	0.75		
Pirenperone	0.5	580 ± 158	462 ± 44 (5)
+ RU24696	0.75		

[a] Figures in parentheses indicate number of animals tested
* Significant difference from appropriate controls $P < 0.025$ unpaired t-test

lated contralateral rotation in DRN lesioned rats similar to that previously reported. In contrast, the piperazine-containing 5-HT agonists MK212 and quipazine were almost devoid of activity.

The rotational behaviour induced by 8-OH-DPAT, RU24696 or 5-MeODMT was not antagonised by either ketanserin or pirenperone, but methysergide caused a significant decrease in turning behaviour (Table 2).

Biochemical studies. Binding studies with 8-OH-DPAT and RU24696 showed that both compounds had significant affinity for [^{3}H]-5-HT (5-HT$_1$) cortical binding sites (pIC50 7.0 and 6.9 respectively), whilst possessing little or no affinity for [^{3}H]-spiroperidol (5-HT$_2$) binding sites (pIC50 <5.0 for both agonists). Binding affinities for 5-HT$_1$ and 5-HT$_2$ binding sites for ketanserin were pIC50 <6.0 and 8.3 respectively and for pirenperone were pIC50 5.0 and 7.5 respectively. In contrast, methysergide had a significant effect on both 5-HT and spiroperidol binding sites (pIC50 7.5 and 8.3 respectively).

Discussion

Unilateral lesions of the cell bodies of 5-HT containing neurones in the DRN causes a denervation supersensitivity of 5-HT receptors located in the substantia nigra (Blackburn et al. 1981).

Following such a lesion in the rat, 5-HT agonists induce contralateral rotational behaviour by a selective action on these receptors. Although binding studies have revealed the existence of different types of 5-HT receptor in brain (Peroutka and Snyder 1979) there has been no attempt previously to define the type of receptor that mediates the rotational response.

The behavioural consequences of an action on central 5-HT receptors (e.g. 5-HT-induced head twitches) are usually believed to be due to an interaction with 5-HT$_2$ binding sites (Leysen et al. 1981). It was of interest therefore to determine which type of receptor was mediating the rotational behaviour.

The agonist studies showed that not all agonists that act at 5-HT receptors are capable of inducing rotational behaviour. In particular, the piperazine-containing agonists quipazine and MK212 were inactive in doses that would be expected to be capable of acting on central 5-HT receptors (Green et al. 1981). In contrast, 5-MeODMT and two other agonists 8-OH-DPAT and RU24696, which are claimed to be selective for 5-HT$_1$ receptors (Arvidsson et al. 1981; Hunt et al. 1981) and confirmed in their selectivity in this study, were all active as inducers of the rotational behaviour. Further, the two antagonists, ketanserin and pirenperone that are claimed to be selective for the 5-HT$_2$ binding site (Leysen et al. 1981) and confirmed to be so in this study, were ineffective as antagonists of the agonist-induced rotational behaviour when used in non-sedative doses. Once again the doses of antagonist used have previously been shown effective in other tests involving an action at central 5-HT$_2$ receptors (Leysen et al. 1981). In contrast to the findings with ketanserin and pirenperone, methysergide was shown to be an effective antagonist. Since the biochemical studies showed that methysergide had significant activity at the 5-HT$_1$ binding sites, it seems reasonable to assume that it is this property that is important in the antagonism of the rotational behaviour.

Thus the general conclusion from this study is that the receptors that mediate 5-HT-induced rotational behaviour in the rat are of the 5-HT$_1$ subtype. Since we have previously

shown that these receptors are located in the substantia nigra (Blackburn et al. 1981), there is a possibility that they could play a role in the physiology and perhaps the pathology of CNS motor functions.

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