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The Discriminative Stimulus Properties of the $5HT_1$ Agonist RU24969

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GARDNER, C. R. *The discriminative stimulus properties of the $5HT_1$ agonist RU24969.* PHARMACOL BIOCHEM BEHAV 33(4) 761–764, 1989.—Using standard operant procedures, rats were trained to discriminate the 5-HT agonist RU24969 (0.5 mg/kg IP) from saline. Stable responding was established and tests of stimulus generalisation and stimulus antagonism were performed with a range of 5-HT receptor ligands. The RU24969 cue was selective as neither 5-HT receptor ligands MK212, DPAT, ipsapirone, GR38032F or the 5-HT releasing agent, fenfluramine nor yohimbine were able to substitute for RU24969 in tests of generalisation. However, TFMPP, CPP and, of particular interest, propranolol substituted for the RU24969 stimulus, although full substitution only occurred with doses that disrupted responding. The RU24969 stimulus was not antagonised by propranolol, metergoline, ritanserin or GR38032F. This pharmacological profile suggests that the RU24969 stimulus is mediated via a subtype of $5\text{-}HT_1$ sites different from $5\text{-}HT_{1A}$ and that propranolol may be an agonist at this site.

Drug discrimination RU24969 5-HT receptors Rats

DRUGS which enhance 5-HT-mediated mechanisms, particularly those which act as agonists at 5-HT receptors, are capable of producing interoceptive stimuli that can be readily discriminated by rats (7, 10, 13, 25). Initial studies with hallucinogens such as lysergic acid diethylamide (LSD) and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) involved a 5-HT receptor now classified as the $5\text{-}HT_2$ subtype (1, 8, 19). With the development of selective ligands for other 5-HT receptor subtypes, drug discrimination studies expanded. It seemed possible that interoceptive stimuli might yield functional correlates for the different putative receptor types that had been classified from binding studies. 1-(3-trifluoromethyl-phenyl)piperazine (TFMPP) serves as a discriminative stimulus in rats and initial evidence suggested the involvement of $5\text{-}HT_{1B}$ sites (4,20). Recently, it has been suggested that both $5\text{-}HT_{1B}$ and $5\text{-}HT_{1C}$ sites could be involved (12). Selective ligands for $5\text{-}HT_{1A}$ sites such as 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT) and 2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-1,2-benzisothiazol-3-(2H) one-1,1-dioxide (TVXQ 7821, ipsapirone) also serve as discriminative stimuli, but with different pharmacological characteristics. Although no specific $5\text{-}HT_{1A}$ antagonist is available, evidence is consistent with the involvement of $5\text{-}HT_{1A}$ sites in the mediation of these stimuli (5, 9, 26, 28). However, generalisation of discriminative stimulus properties of both DPAT and ipsapirone to yohimbine led Winter (24) to question this pharmacological specificity.

RU24969 (5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H indole) is selective for $5\text{-}HT_1$ sites (18), but shows little selectivity between the $5\text{-}HT_1$ subsites (21, 24, 27). It induces a behavioural syndrome in rodents with prominent hyperactivity which is not identical to that of the putative $5\text{-}HT_{1A}$ agonist DPAT (14,15). However, RU24969 has been shown to induce reciprocal forepaw treading in rats which is thought to result from activation of $5\text{-}HT_{1A}$ sites (21). Thus, RU24969 may produce some behavioural effects by interacting with different $5\text{-}HT_1$ subsites. In drug discrimination studies RU24969 substituted for the TFMPP discriminative stimulus, but DPAT did not (11,12), whilst the DPAT stimulus does not generalise to RU24969 (28). Interoceptive stimuli induced by RU24969, as indicated by generalisation studies with other training drugs, appear not to involve $5\text{-}HT_{1A}$ sites. In order to further establish the characteristics of discriminative stimuli induced by RU24969, rats were trained to discriminate the drug from saline and the pharmacology of this discrimination was investigated.

METHOD

Subjects

Male hooded Lister rats (Olac, Bicester, UK) were used in all these studies. At the beginning of training rats were 180–220 g and they continued performing these experiments as long as their baseline responding was stable. Therefore, most pharmacological studies were performed with rats weighing 300–500 g.

Animals were housed in pairs in a colony room maintained at 22°C and with controlled humidity, on an 8.00–18.00, 18.00–8.00 light-dark cycle. Water was continuously available in the home cages, but food was restricted to 80% of that consumed by ad lib fed controls. The rats were fed approximately 4 hr after the operant session. Food was scattered on the floor of the home cage to minimise competition for food between members of each pair.

Nine rats were used for the cue. Each member of a pair was

trained on the same cue drug, and both were tested in different operant chambers, but at the same time.

Apparatus

The behavioural apparatus consisted of identical standard Skinner boxes (Camden Instruments, London) each with two retractable levers on one wall, with the food dispensing magazine centrally between them. Each apparatus was housed in a light-proof, sound-attenuated, fan-ventilated chamber.

Operation of the behaviour schedules and recording of data were achieved using microcomputers (Acorn series II) via appropriate interfacing (Camden Instruments). Levers were retracted when the rats were placed in the apparatus and each session began when both levers were simultaneously presented.

Discrimination Training

Rats were trained to discriminate between the effect of vehicle and that of RU24969 0.5 mg/kg IP 30 min prior to testing. Rats were magazine trained and shaped to press the lever for food reinforcement (45 mg pellets, Camden Instruments). Then they were trained to respond on one of the levers following cue drug injection and on the other lever following administration of vehicle (demineralised water, 2 ml/kg). A food pellet was delivered after every 20th press (FR20) on the correct lever. Responses on the incorrect lever (i.e., drug lever after vehicle injection or vehicle lever after cue drug injection) were recorded, but were not reinforced with food pellet reward.

The drug lever was randomly allocated on the right side of the food magazine for half the rats and on the left side for the other half. The position of the drug and vehicle levers remained constant for each rat for all subsequent sessions. The sequence of drug-vehicle injection was different throughout groups of rats to control for a possible olfactory cue and a quasirandom (vehicle-drug-drug-vehicle-vehicle and drug-vehicle-vehicle-drug-drug) sequence of testing was used for each successive two week, Monday to Friday test block.

Training criterion was reached when the number of presses prior to receiving the first food pellet (FFP) was <24 for the prior two sessions of both drug and vehicle training. This criterion was maintained throughout drug testing as an index of stable baseline responding. In the majority of cases the FFP was 20 under these fully trained conditions.

Drug Testing

Rats reaching the criterion level of performance were repeatedly used in generalisation and antagonism testing. At least one vehicle and one cue drug response at criterion level was required between each such test. Any given drug/dose combination was allocated randomly to rats as they became available for testing. Where necessary the route of vehicle administration was changed if a test compound to be administered instead of the cue drug in generalisation studies was to be given by a different route from the cue drug. In antagonism studies, when both test compound and cue drug are given, an appropriate vehicle injection was given as well as the cue drug in control tests. Vehicle and cue drug test sessions were 10 min in duration, whilst tests with noncue drugs were 5 min in duration. Following the choice in the test sessions reward was available on an FR20 schedule on the lever of choice.

Drugs

All drugs were dissolved or suspended in 0.9% saline and administered intraperitoneally (1 ml/kg) 30 min prior to testing with the exception of yohimbine which was given 15 min prior to test. All drugs were sonicated and continuously stirred until used.

We acknowledge the generous gifts of metergoline (Farmitalia, Italy) and GR38032F (Glaxo Laboratories, UK). RU24969, ritanserin and ipsapirone were synthesised by Roussel chemists. All other compounds were obtained commercially.

RESULTS

All rats received 56 training sessions with only the treatment-appropriate lever rewarded. Following this, when both levers were available, rats required a mean of 26.2 trials (range 22 to 32) to reach criterion level of choice on both drug and vehicle-appropriate levers.

In generalisation studies, RU24969, TFMPP and 1-(3-chlorophenyl)piperazine (CPP) substituted in a dose-related manner for the discriminative stimulus produced by 0.5 mg/kg RU24969. Doses of TFMPP and CPP showing full substitution were associated with disruption of performance and thus an incidence of rats not reaching criterion (20 presses on one lever) in the test session (Table 1). MK212 showed partial substitution, but only at a dose (1 mg/kg) which markedly disrupted responding. Fenfluramine, DPAT and ipsapirone did not substitute despite some disruption of performance at highest doses tested. GR38032F and yohimbine did not substitute at the doses tested (0.01, 0.1, 0.5 mg/kg and 2 mg/kg respectively). ±Propranolol was originally used as a potential antagonist and was found to be inactive in this respect. A combination of 0.5 mg/kg RU24969 and 20 mg/kg propranolol resulted in total abolition of responding. However, propranolol substituted for the RU24969 stimulus in a dose-related manner with a maximum 89% at 20 mg/kg.

Other 5-HT antagonists metergoline, ritanserin and GR38032F did not reduce drug-appropriate responding when combined with 0.5 mg/kg RU24969 (Table 2). Doses of metergoline and ritanserin used were fully effective in antagonising other 5-HT receptor-mediated responses in this strain of rat in our laboratories.

DISCUSSION

The lack of substitution for the RU24969 stimulus with MK212, together with the lack of antagonism by metergoline and ritanserin, suggest that 5-HT_2 receptors are not involved. This is consistent with the negligible affinity of RU24969 for 5-HT_2 binding sites (18). It is interesting that metergoline, which also acts at 5-HT_1 receptors (23,24), did not block the RU24969 stimulus. The lack of interaction of the specific 5-HT_3 receptor antagonist GR38032F (2) with the RU24969 stimulus suggests also a lack of involvement of 5-HT_3 receptors. The antagonism study was limited by compound supply, but the chosen dose is at the high end of those having pharmacological effects in vivo (3).

Within the 5-HT_1 class of sites the lack of substitution by DPAT and ipsapirone tends to exclude the involvement of 5-HT_{1A} sites. Certainly, it establishes that the discriminative stimuli produced by RU24969 and DPAT are different. The difference is further underlined by the lack of substitution by yohimbine for the RU24969 stimulus, whereas it substitutes for both DPAT and ipsapirone (29). The dose of yohimbine used in this study is close to the ED_{50} for substitution for DPAT or ipsapirone (29) and is limited by disruption of performance by yohimbine at higher doses in our experience. Additionally, propranolol, in common with some other β-adrenoreceptor blockers, can antagonize reciprocal forepaw treading and hypothermia induce by DPAT (27). The DPAT stimulus is blocked by pindolol and alprenolol (28). These actions presumably relate to the affinity of these agents for 5-HT_{1A} sites (22). However, propranolol did not antagonise the RU24969 stimulus, but substituted for it.

Propranolol has also similar affinity for 5-HT_{1B} sites (6,17) and it is possible that the RU24969 stimulus results from activation of

TABLE 1

STIMULUS GENERALISATION STUDIES USING RATS TRAINED TO DISCRIMINATE RU24969 (0.5 mg/kg) FROM SALINE

Agent	Dose mg/kg	N*	Drug-Appropriate Responding Number	Percentage
RU24969	0.5	10/10	9/10	90
	0.25	7/7	4/7	57
	0.1	6/6	1/6	17
TFMPP	2	3/7	3/3	100
	1	8/12	7/8	87.5
	0.75	5/7	5/5	100
	0.5	11/11	8/11	73
	0.35	5/6	3/5	60
	0.2	12/12	2/12	17
CPP	2	2/6	2/2	100
	1	8/16	4/8	50
	0.75	7/10	6/7	86
	0.5	7/7	2/7	29
Fenfluramine	2	12/15	3/12	25
	1	10/10	0/10	0
MK212	1	4/14	2/4	50
	0.5	11/14	1/11	9
	0.2	5/5	0/5	0
DPAT	0.5	2/7	0/2	0
	0.2	7/7	0/7	0
Ipsapirone	10	5/12	0/5	0
	5	5/8	0/5	0
GR38032F	0.5	7/7	0/7	0
	0.1	6/6	0/6	0
	0.01	5/5	1/5	20
± Propranolol	20	9/14	8/9	89
	10	7/8	4/7	57
	5	8/8	4/8	50
	2	3/3	1/3	33.3
Yohimbine	2	10/10	0/10	0

*N = number of rats responding/number to receive drug.

TABLE 2

STIMULUS ANTAGONISM STUDIES USING RATS TRAINED TO DISCRIMINATE RU24969 (0.5 mg/kg) FROM SALINE

Agent	Dose mg/kg	N*	Drug-Appropriate Responding Number	Percentage
Saline		6/6	6/6	100
Metergoline	10	4/4	4/4	100
	5	8/8	6/8	75
	2	4/4	4/4	100
± Propranolol	20	0/4		
	10	7/7	7/7	100
Ritanserin	10	6/6	6/6	100
GR38032F	0.5	6/6	6/6	100

*N = number of rats responding/number to receive drugs.

these sites and that propranolol is an agonist at these sites. The pharmacology of the RU24969 stimulus is very similar to that found with rats trained to discriminate TFMPP (4, 11, 20). Discriminative stimuli induced by the two agents cross generalise and CPP substitutes for both. In the studies of Glennon *et al.* (11,20) and Cunningham and Appel (4) in rats trained to discriminate TFMPP, RU24969 and TFMPP substituted for the cue with ED_{50} values of 0.17, 0.28 mg/kg and 0.23, 0.25 mg/kg respectively. CPP was less potent (0.47, 0.38 mg/kg). The relative potencies of these drugs in substituting for the RU24969 cue was very similar; approximate ED_{50} values being 0.21 mg/kg for RU24969, 0.32, mg/kg for TFMPP and 0.65 mg/kg for CPP. This further supports the similarity between the discriminative stimuli induced by TFMPP and RU24969. As with the RU24969 stimulus here, propranolol (and pindolol) substitute for TFMPP (12). It has been previously suggested that pindolol may have mixed agonist-antagonist properties at central 5-HT receptors, on the basis of 5-HT turnover measurements (16). It seems likely that RU24969 and TFMPP produce discriminative stimuli via the same mechanism. Although the majority of evidence suggests that 5-HT_{1B} sites represent this mechanism (10,20), the substitution by mesulergine for TFMPP is not consistent with this view (12). Mesulergine is selective for 5-HT_{1C} binding sites implying that these sites might be involved in the TFMPP discriminative stimulus. Further pharmacological studies will be required to closely characterise the receptor sites activated that lead to the discriminative stimulus of RU24969. This would also help to determine the functional significance of different 5-HT binding sites.

It seems surprising that the 5-HT releasing agent fenfluramine does not substitute for the RU24969 stimulus. It could be that the 5-HT is released differentially in different 5-HT-mediated neurotransmissions and tends to lead to the activation of a 5-HT receptor type other than that involved in the RU24969 stimulus. Alternatively, as was suggested by Tricklebank *et al.* (28) for the DPAT stimulus, there may be functional interactions resulting from activation of different 5-HT receptors such that the discriminative stimulus is distinct when only the mediating receptor is activated, but less distinct if several receptors are activated.

In summary, RU24969 produces a discriminative stimulus distinct from that of DPAT, but similar to that of TFMPP. 5-HT_2, 5-HT_3 and 5-HT_{1A} receptor activation does not seem to underlie this discriminative stimulus. It seems likely that another subtype of 5-HT_1 receptors (5-HT_{1B} and/or 5-HT_{1C}) is involved in the generation of the RU24969-induced discriminative stimulus.

REFERENCES

1. Bradley, P. B.; Engel, G.; Feniuk, W.; Fozard, J. R.; Humphrey, P. A.; Middlemiss, D. M.; Mylecharane, E. J.; Richardson, B. P.; Saxena, P. R. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25:563–576; 1986.
2. Butler, A.; Hill, J. M.; Ireland, S. J.; Jordan, C. C.; Tyers, M. B. Pharmacological properties of GR38032F, a novel antagonist at 5-HT_3 receptors. Br. J. Pharmacol. 94:397–412; 1988.
3. Costall, B.; Domeney, A. M.; Naylor, R. J.; Tyers, M. B. Effects of the 5-HT receptor antagonist, GR38032F, on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. Br. J. Pharmacol. 92:881–894; 1987.
4. Cunningham, K. A.; Appel, J. B. Possible 5-hydroxytryptamine-1 (5-HT_1) receptor involvement in the stimulus properties of 1-(m-

trifluoromethylphenyl) piperazine (TFMPP). J. Pharmacol. Exp. Ther. 237:369–377; 1986.
5. Cunningham, K. A.; Callahan, P. M.; Appel, J. B. Discriminative stimulus properties of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT): implications for understanding the actions of novel anxiolytics. Eur. J. Pharmacol. 138:29–36; 1987.
6. Engel, G.; Gothert, M.; Hoyer, D.; Schlicker, E.; Hillenbrand, K. Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. Naunyn Schmiedebergs Arch. Pharmacol. 332:1–7; 1986.
7. Friedman, R.; Barrett, R. J.; Sanders-Bush, E. Additional evidence that L-5-hydroxytryptophan discrimination models a unique serotonin receptor. Psychopharmacology (Berlin) 80:209–213; 1983.
8. Glennon, R. A. Involvement of serotonin in the action of hallucinogenic agents. In: Green, A. R., ed. Neuropharmacology of serotonin. Oxford: Oxford University Press; 1985:253–280.
9. Glennon, R. A. Discriminative stimulus properties of the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). Pharmacol. Biochem. Behav. 25:135–139; 1986.
10. Glennon, R. A. Site selective serotonin agonists as discriminative stimuli. Psychopharmacol. Ser. 4:15–31; 1988.
11. Glennon, R. A.; McKenney, J. D.; Young, R. Discriminative stimulus properties of the serotonin agonist 1-(3-trifluoromethylphenyl) piperazine (TFMPP). Life Sci. 35:1475–1480; 1984.
12. Glennon, R. A.; Pierson, M. E.; McKenney, J. D. Stimulus generalisation of 1-(3-trifluoromethylphenyl) piperazine (TFMPP) to propranolol, pindolol and mesulergine. Pharmacol. Biochem. Behav. 29:197–199; 1988.
13. Glennon, R. A.; Young, R.; Rosecrans, J. A. Antagonism of the effects of the hallucinogen DOM and the purported 5-HT agonist quipazine by 5-HT_2 antagonists. Eur. J. Pharmacol. 91:189–196; 1983.
14. Goodwin, G. M.; Green, A. R. A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists of 5-HT_1 and 5-HT_2 receptors. Br. J. Pharmacol. 84:743–753; 1985.
15. Green, A. R.; Guy, A. P.; Gardner, C. R. The behavioural effects of RU24969, a suggested 5-HT_1 receptor agonist, in rodents and the effect on the behaviour of treatment with antidepressants. Neuropharmacology 23:655–661; 1984.
16. Hjorth, S.; Carlsson, A. Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptors? Eur. J. Pharmacol. 129:131–138; 1986.
17. Hoyer, D.; Engel, G.; Kalkman, H. O. Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH DPAT, (−) [^{125}I]-iodocyanopindolol, [^{3}H] mesulergine and [^{3}H]ketanserin. Eur. J. Pharmacol. 118:13–23; 1985.
18. Hunt, P.; Oberlander, C. The interaction of indole derivatives with the serotonin receptor and non-dopaminergic circling behaviours. In: Haber, B., ed. Serotonin—Current aspects of neurochemistry and function. New York: Plenum; 1981:547–561.
19. Leff, P.; Martin, G. R. The classification of 5-hydroxytryptamine receptors. Med. Res. Rev. 8:187–202; 1988.
20. McKenney, J. D.; Glennon, R. A. TFMPP may produce its stimulus effects via a 5-HT_{1B} mechanism. Pharmacol. Biochem. Behav. 24:43–47; 1986.
21. Peroutka, S. J. Selective labelling of 5-HT_{1A} and 5-HT_{1B} binding sites in bovine brain. Brain Res. 344:167–171; 1985.
22. Peroutka, S. J. Selective interaction of novel anxiolytics with 5-hydroxytryptamine receptors. Biol. Psychiatry 20:971–979; 1985.
23. Peroutka, S. J. Pharmacological differentiation and characterisation of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} binding sites in rat frontal cortex. J. Neurochem. 47:529–540; 1986.
24. Sills, M. A.; Wolfe, B. B.; Frazer, A. Determination of selective and non-selective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. J. Pharmacol. Exp. Ther. 231:480–487; 1984.
25. Spencer, D. G.; Glaser, T.; Traber, J. Serotonin receptor subtype mediation of the interoceptive discrimative stimuli induced by 5-methoxy-N,N-dimethyltryptamine. Psychopharmacology (Berlin) 93: 158–166; 1987.
26. Spencer, D. G.; Traber, J. The interoceptive stimuli induced by the novel putative anxiolytic TVXQ 7821: Behavioural evidence for the specific involvement of serotonin 5-HT_{1A} receptors. Psychopharmacology (Berlin) 91:25–29; 1987.
27. Tricklebank, M. D.; Middlemiss, D. N.; Neil, J. Pharmacological analysis of the behavioural and thermoregulatory effects of the putative 5-HT_1 receptor agonist RU24969, in the rat. Neuropharmacology 25:877–886; 1986.
28. Trickebank, M. D.; Neill, J.; Kidd, E. J.; Fozard, J. R. Mediation of the discriminative stimulus properties of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) by the putative 5-HT_{1A} receptor. Eur. J. Pharmacol. 133:47–56; 1987.
29. Winter, J. C. Generalisation of the discriminative stimulus properties of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and ipsapirone to yohimbine. Pharmacol. Biochem. Behav. 29:193–195; 1988.