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The putative 5-HT_1 receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor

DEREK N. MIDDLEMISS, *Merrell Dow Research Institute, Strasbourg Centre, 16, rue d'Ankara 67084 Strasbourg-Cedex, France*

The putative central 5-HT receptor agonist, 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate (RU 24969), was found to be a potent inhibitor of the continuous K^+ evoked efflux of [^{3}H]5-HT from superfused rat frontal cortex slices (pD_2 7·45). The effects of RU 24969 were attenuated by the putative 5-HT autoreceptor antagonists, methiothepin, quipazine and (−)-propranolol but not by the α_2-adrenoceptor antagonist, idazoxan. It is concluded that RU 24969 inhibits K^+ evoked efflux of [^{3}H]5-HT from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. Moreover, since RU 24969 potently displaced ligand binding to the 5-HT_1 and 5-HT_{1B} recognition sites but was only weakly active at the 5-HT_2 receptor, the results lend support to the claim for a pharmacological resemblance between the 5-HT autoreceptor and the 5-HT_1 recognition site and in particular the low affinity 5-HT_{1B} subtype.

5-Methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole succinate (RU 24969) is a novel piperidinyl indole which decreases 5-hydroxyindoleacetic acid (5-HIAA) levels in rat brain after peripheral administration (Euvrard & Boissier 1980). The effect is consistent with agonist activity at central 5-hydroxytryptamine (5-HT) receptors, an interpretation strengthened by the observation that RU 24969 is a potent and selective displacer of [^{3}H]5-HT receptor binding (Hunt & Oberlander 1981).

The reduction in brain 5-HIAA concentrations could reflect either an inhibition of 5-HT release following post-synaptic receptor stimulation and a neuronal feedback loop, or a direct interaction of RU 24969 with the 5-HT autoreceptor. This latter possibility has been investigated using inhibition of K^+ evoked efflux of [^{3}H]5-HT from rat brain slices as an index of autoreceptor activity. In addition, the potency of RU 24969 to displace ligand binding to the various central 5-HT receptor subtypes has been measured by receptor binding techniques. The results indicate that RU 24969 is a potent 5-HT autoreceptor agonist with marked activity at the 5-HT_{1B} subtype of the 5-HT_1 recognition site.

Materials and methods

Superfusion studies. The effect of drugs on K^+ evoked efflux of [^{3}H]5-HT was studied using the continuous stimulation technique of Frankhuyzen & Mulder (1982) adapted for rat frontal cortex slices as described by Middlemiss (1984a, b). Briefly, the frontal cortices from two male Sprague-Dawley rats (200–250 g) were chopped in two directions at 250 μm intervals and incubated with 0·1 μM [^{3}H]5-HT in the presence of 10 μM pargyline in Krebs-Henseleit buffer oxygenated with 95% O_2–5% CO_2 at 37 °C. After 15 min the slices were washed and 50 μl aliquots (about 20 mg tissue) were transferred to each chamber of a superfusion apparatus and superfused at 0·38 ml min^{-1} with Krebs buffer. The superfusion solution contained 3·2 μM paroxetine to prevent the neuronal uptake of [^{3}H]5-HT. After 30 min of superfusion, some of the slices were exposed to Krebs buffer containing elevated K^+ ions (25 mM) for 16 min (i.e. t = 30–46 min) and then 18 successive 4 min fractions of the superfusate (continuously superfused with either Krebs or 25 mM K^+ Krebs) were collected (i.e. t = 46–118 min). At the end of the experiment the radioactivity in the slices and in each fraction was determined by liquid scintillation counting.

Cumulative dose-responses to RU 24969 were constructed using four concentrations of agonist (30 nM to 1 μM) with a 16 min interval between each increase in agonist concentration. Experiments with antagonists were carried out by adding drug to the superfusion media (basal and elevated K^+) at t = 30 min, drug was then present throughout the superfusion. Since under these conditions RU 24969, at concentrations up to 1 μM, did not affect the baseline efflux of tritium, basal efflux was taken as the release in the absence of added agonist with or without added antagonist as appropriate. The results were calculated as described by Frankhuyzen & Mulder (1982). Determination of apparent pA_2 values was performed as described by

Schlicker & Göthert (1981). Statistical significance was assessed using Student's *t*-test.

Receptor binding studies. 5-HT_1, 5-HT_{1B} and 5-HT_2 receptor binding studies were carried out, with minor modification, as described by Middlemiss & Fozard (1983). The frontal cortices of male Sprague-Dawley rats (200–250 g) were dissected, frozen in liquid nitrogen and stored at −20 °C until needed. Tissue from 4–8 rats was pooled and homogenized in 70 vol Tris-HCl buffer (50 mM, pH 7·7) using a Kinematica Polytron (setting 2/3 max speed, 20 s). The homogenate was centrifuged (36 500*g* for 10 min), the pellet rehomogenized in the same volume of buffer and the process repeated twice. Between the second and third centrifugations, the tissue homogenate was incubated at 37 °C for 10 min to remove endogenous 5-HT. The final pellet was suspended in the same volume of Tris buffer (50 mM, pH 7·7) containing 10 μM pargyline, 5·7 mM $CaCl_2$ and 0·1% ascorbic acid. This suspension was incubated for 10 min at 37 °C and then stored on ice until used in the binding assay.

Tissue homogenate (0·7 ml), radioactive ligand (0·1 ml) and the appropriate concentrations of drug (0·1 ml) together with buffer to a final volume of 1 ml were incubated at 37 °C for 15 min. Incubations were terminated by rapid filtration through Whatman GF/B filters followed by three 5 ml washes with ice-cold Tris-HCl buffer (50 mM, pH 7·0). Radioactivity was measured after extraction into Aquasol-2 (NEN) at an efficiency at 45–50%. The radioligands used were: 5-HT_1 and 5-HT_{1B}—2 nM [^{3}H]5-HT; 5-HT_2—1 nM [^{3}H] ketanserin (Leysen et al 1982). 5-HT_{1B} binding was carried out in the presence of 1 μM spiperone. Non-specific binding was defined using 10 μM 5-methoxytryptamine for 5-HT_1 and 5-HT_{1B} binding and 1 μM mianserin for 5-HT_2 binding. Specific binding represented 65–75% of total binding for both 5-HT_1 and 5-HT_2 binding and 40–50% for 5-HT_{1B} binding.

Drugs used. [^{3}H]5-Hydroxytryptamine creatinine sulphate (28·3 Ci mmol^{-1}, New England Nuclear), [^{3}H]-ketanserin HCl (64·6 Ci mmol^{-1}, New England Nuclear), RU 24969 succinate (Roussel-Uclaf), pargyline HCl (Abbott), paroxetine HCl (Ferrosan), methiothepin maleate (Roche), quipazine maleate (Miles), (−)-propranolol HCl (ICI), idazoxan HCl (Reckitt and Colman), spiperone (Janssen), 5-hydroxytryptamine creatinine sulphate (Sigma).

Results

[^{3}H]5-HT release studies from rat frontal cortex. A continuous elevated (25 mM) K^+ concentration in the superfusion medium caused an increased efflux of tritium from preloaded slices of the rat frontal cortex. Baseline efflux at t = 54 min was 0·64 ± 0·04% tissue stores min^{-1} (mean ± s.e.m., n = 6). Efflux in the presence of 25 mM K^+ Krebs was 2·14 ± 0·13% tissue stores min^{-1} (n = 6) at t = 54 min. The additional overflow induced by the elevated K^+ concentration was completely prevented by the omission of Ca^{2+} from the superfusion medium (data not shown).

When added in a cumulative fashion to the superfusion medium, RU 24969 in the concentration range 30 nM to 1 μM caused a concentration-related inhibition of the K^+ evoked efflux of tritium (Fig. 1). The maximum effect, observed at 1 μM RU 24969, represented a reduction in K^+ evoked efflux of 59 ± 4% (n = 6). The apparent affinity (pD_2) calculated from these experiments assuming a maximum effect at 1 μM RU 24969 was 7·45 ± 0·20 (n = 6).

Addition of the putative 5-HT autoreceptor antagonists methiothepin (1 μM) (Göthert 1980) and quipazine (10 μM) (Schlicker & Göthert 1981) to the superfusion medium caused a significant enhancement of the K^+ evoked efflux of tritium (efflux rate at t = 54 min: methiothepin, 3·10 ± 0·15 (n = 6), $P < 0·001$ with respect to controls; quipazine, 2·77 ± 0·15 (n = 6), $P < 0·01$ with respect to controls). In contrast the putative 5-HT autoreceptor antagonist (−)-propranolol (1 μM) (Middlemiss 1984a) was without effect on the K^+ evoked release of tritium (efflux rate at t = 54 min, 2·19 ± 0·09 (n = 6, NS). In the presence of the stated concentrations of these three putative 5-HT autoreceptor antagonists, the concentration-effect curve for RU 24969 was shifted to the right (Fig. 1). The apparent pA_2 values calculated from the data shown in Fig. 1 were found to be 6·27 (methiothepin), 5·60 (quipazine) and 6·45 ((−)-propranolol).

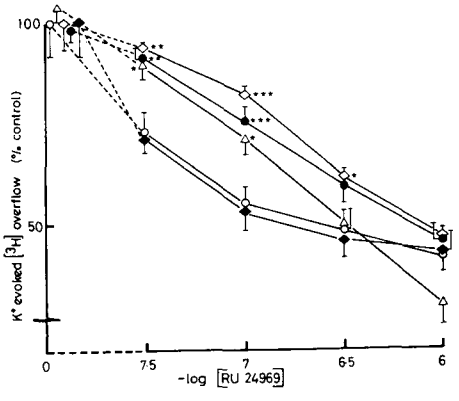

FIG. 1. Concentration-effect curves for RU 24969 (○) and RU 24969 in the presence of 1 μM methiothepin (△), 10 μM quipazine (◇), 1 μM (−)-propranolol (●) and 1 μM idazoxan (◆). Cumulative dose response curves to RU 24969 were carried out by increasing the concentration of RU 24969 stepwise at 16 min intervals. Antagonists were added at t = 30 min and were present throughout the rest of the superfusion. Each determination is the mean ± s.e.m. of 5 or 6 separate experiments. Statistical significance with respect to control activity of RU 24969 (% inhibition in the absence of added antagonist) was assessed using Student's *t*-test (*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$).

The addition of the selective α_2-adrenoceptor antagonist, idazoxan (1 μM), to the superfusion medium caused no change in either the basal or K^+ evoked release rates of tritium (basal and K^+ evoked efflux rates at t = 54 min were respectively 0·58 ± 0·03 and 2·31 ± 0·14 (n = 6)); similarly, the concentration-effect curve of RU 24969 was unaffected by idazoxan (Fig. 1).

Receptor binding studies. RU 24969 and 5-HT were potent inhibitors of ligand binding to both the 5-HT_1 and 5-HT_{1B} recognition sites but were only weakly active at the 5-HT_2 receptor (Table 1).

Table 1. Comparison of the activity of RU 24969 and 5-HT at the 5-HT autoreceptor and at central 5-HT recognition sites.

	RU 24969	5-HT
Release studies		
pD_2	7·45 ± 0·20	7·34 ± 0·05
Maximum inhibition (at 1 μM)	59 ± 4	68 ± 3
Apparent pA_2		
Methiothepin	6·27	6·90
Quipazine	5·60	5·90
(−)Propranolol	6·45	6·67
Idazoxan	No effect at 1 μM	N.D.
Receptor binding studies (pIC50)		
5-HT_1	8·02 ± 0·07	8·25 ± 0·01
5-HT_{1B}	8·18 ± 0·10	7·92 ± 0·05
5-HT_2	5·29 ± 0·03	5·50 ± 0·02

Release data for RU 24969 were derived from Fig. 1 as described in text. Release data with 5-HT as agonist are taken from Middlemiss (1984a, b). Receptor binding data are expressed as pIC50 = $-\log_{10}$ concentration of drug to inhibit specific binding by 50% and are the mean ± s.e.m. of at least 3 independent experiments. N.D. = not determined.

Discussion

To facilitate discussion of the effects of RU 24969, the key observations are compared with those reported earlier for 5-HT in Table 1. The K^+ evoked efflux of [^{3}H]5-HT from slices of rat frontal cortex was inhibited in a concentration dependent manner by RU 24969. The pD_2 for this effect was 7·45 which is close to the previously reported value for 5-HT itself (7·34) determined using an identical technique (Middlemiss 1984a). The maximum inhibition of 59% seen with RU 24969 was not significantly different from that obtained with 5-HT in previous studies (68%—Table 1). The inhibitory effects of both RU 24969 and 5-HT were attenuated by the putative 5-HT autoreceptor antagonists methiothepin, quipazine and (−)-propranolol and in the case of RU 24969 were unaffected by the α_2-adrenoceptor antagonist idazoxan (Chapleo et al 1981; Galzin et al 1984) at a concentration of 1 μM (Table 1). The lack of effect of idazoxan suggests that the inhibitory effect of RU 24969 is not mediated through the α_2-adrenoceptor which is known to be present on 5-HT nerve terminals and to inhibit release when activated (Göthert et al 1981; Galzin et al 1984). The similarity of the apparent pA_2 values for the putative 5-HT autoreceptor antagonists against RU 24969 when compared with their apparent pA_2 values against 5-HT (Table 1) suggests that inhibition is mediated via the 5-HT autoreceptor. This conclusion is further strengthened by the observation that the inhibitory effects of RU 24969 persist unchanged in the presence of 0·1 μM tetrodotoxin (data not shown), a finding which is consistent with a direct effect of RU 24969 at the 5-HT autoreceptor (Göthert 1982).

The potent agonist effects of RU 24969 at the 5-HT autoreceptor would, if manifested in-vivo, be adequate to explain the activity of this drug in reducing 5-HIAA in rat brain (Euvrard & Boissier 1980) although a postsynaptic effect involving a neuronal feedback loop cannot obviously be ruled out. The relation of this agonist activity at the 5-HT autoreceptor to the behavioural effects seen with RU 24969 in rodents (Hunt & Oberlander 1981; Gardner & Guy 1983; Green et al 1984; Tricklebank 1984) is not at present known.

The present data also bear on the proposed relation between the [^{3}H]5-HT binding site and the 5-HT autoreceptor in the rat brain. Initial studies using a variety of 5-HT agonists at the 5-HT autoreceptor suggested that the pharmacological specificity of this receptor may bear some resemblance to that of the 5-HT_1 recognition site labelled by [^{3}H]5-HT (Martin & Sanders-Bush 1982; Göthert & Schlicker 1983). The subsequent extension of these studies to antagonists has led to the suggestion that potency at the 5-HT autoreceptor correlates better with affinity for a low affinity component of [^{3}H]5-HT binding (Engel et al 1983). The present results with RU 24969 which has strong activity at the low affinity, 5-HT_{1B}, subtype of the 5-HT_1 recognition site would be consistent with this latter view. This interpretation is supported by the fact that another putative 5-HT_1 receptor agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin, has marked potency and selectivity for the high affinity, 5-HT_{1A} subtype of the 5-HT_1 recognition site yet is inactive at the 5-HT autoreceptor (Middlemiss 1984b).

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Comparative potencies of calcium channel antagonists and antischizophrenic drugs on central and peripheral calcium channel binding sites

RÉMI QUIRION*, F. LAFAILLE, N. P. V. NAIR, *Douglas Hospital Research Centre and Dept. of Psychiatry, Faculty of Medicine, McGill University, 6875 LaSalle Blvd, Verdun, Qué. H4H 1R3 Canada*

Dihydropyridines are potent agents on [^{3}H]nitrendipine binding sites in heart and brain membranes. Like the phenylalkylamines, they are slightly more active on heart than on brain [^{3}H]nitrendipine binding sites. On the other hand, the diphenylalkylamines, the diphenylpiperazines and the antischizophrenic drugs of the diphenylbutylpiperidine type are more potent on brain [^{3}H]nitrendipine binding sites. The findings suggest tissue heterogeneity of [^{3}H]nitrendipine binding sites and the possible development of diphenylbutylpiperidine-diphenylbutylpiperazine analogues that could selectively act on brain calcium channel antagonist binding sites.

Organic calcium channel antagonists have emerged as major therapeutic agents in the treatment of various cardiovascular disorders (Antman et al 1980; Stone et al 1980). Various classes of calcium antagonists have been developed including the 1,4-dihydropyridines (e.g. nifedipine, nitrendipine), the phenylalkylamines (e.g. verapamil), the diphenylbutylpiperazines (e.g. lidoflazine) and the diphenylalkylamines (e.g. phenylamine) (Janis & Triggle 1983: Janis & Scriabine 1983).

Recently, some of these drugs have been radioactively labelled and the existence of specific binding sites for the 1,4-dihydropyridines (Bellemann et al 1981; Bolger et al 1983; Ehlert et al 1982; Ferry & Glossmann 1982a; Janis et al 1982; Murphy & Snyder 1982; Ferry et al 1983; Gould et al 1984; Janis & Triggle 1984), verapamil and derivatives (Reynolds et al 1983; Ferry et al 1984; Galizzi et al 1984; Goll et al 1984) and diltiazem (Glossmann et al 1983) has been described in various tissues. It is currently believed that non-dihydropyridine calcium channel antagonists act at one or more sites allosterically linked to the 1,4-dihydropyridine sites (Ehlert et al 1982; Ferry & Glossmann 1982b; Glossman et al 1982; Janis et al 1982; Yamamura et al 1982; Murphy et al 1983; Miller & Freedman 1984). Autoradiographic studies have shown that [^{3}H]1,4-dihydropyridines (Murphy et al 1982; Cortes et al 1983; Quirion 1983) and [^{3}H]desmethoxyverapamil (Ferry et al 1984) binding sites are similarly distributed in various regions of the brain. These sites are mainly concentrated in the dentate gyrus, superficial layers of the cortex and external plexiform layers of the olfactory bulb (Quirion 1983).

It has been reported that antischizophrenic drugs of the diphenylbutylpiperidine type can inhibit smooth muscle contraction, possibly by an action on calcium channels (Quintana 1978; Spedding 1982). Moreover, Gould et al (1983) have recently demonstrated that these drugs are potent inhibitors of [^{3}H]nitrendipine binding sites in rat brain membranes. It suggests that certain actions of these antischizophrenic drugs could be related to a blockade of calcium channels. We report here that these drugs appear to interact preferentially with brain [^{3}H]nitrendipine binding sites. They are less potent in cardiac membrane preparations. It suggests that sites labelled by [^{3}H]nitrendipine are slightly different sites in these two tissues. Moreover, calcium channel antagonists structurally related to the diphenyl-

* Correspondence.