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The 5-HT_1 receptor agonist RU-24969 decreases 5-hydroxytryptamine (5-HT) release and metabolism in the rat frontal cortex in *vitro* and *in vivo*

M.P. Brazell[1], C.A. Marsden[2], A.P. Nisbet & C. Routledge

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH

1 K^+-stimulated release of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) from rat frontal cortex slices was decreased by the 5-HT receptor agonists 5-methoxy-n_1N-dimethyltryptamine and 5-methoxy-3(1,2,3,6,-tetrahydro-4-pyrindinyl)-1H-indole (RU-24969) (1×10^{-5}M).

2 RU-24969 (10 mg kg^{-1}, i.p.) decreased extracellular 5-HT and its metabolite 5-hydroxyindoleacetic acid measured *in vivo* by use of intracerebral dialysis combined with high performance liquid chromatography and electrochemical detection.

3 The decrease in extracellular 5-hydroxyindoleacetic acid *in vivo* after RU-24969 (10 mg kg^{-1}, i.p.) was also observed by *in vivo* voltammetry.

4 The non-selective 5-HT antagonist metergoline prevented the RU-24969-induced decrease in 5-HT release and metabolism *in vivo* while the 5-HT_2 receptor antagonist R-55669 (ritanserin) did not.

6 The results support the view that RU-24969 stimulates a 5-HT_1 receptor that is involved in the autoregulation of 5-HT release and metabolism.

Introduction

Recently, a number of reportedly selective 5-hydroxytryptamine (5-HT) receptor agonists have been developed. One of these, 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU-24969), appears from binding (Hunt & Oberlander, 1981) and behavioural (Gardner & Guy, 1983; Tricklebank, 1984, Green *et al.*, 1984) studies to be selective for 5-HT_1 receptors. RU-24969 decreases 5-HT turnover as shown by a decrease in brain 5-HIAA (Euvrard & Boissier, 1980) and inhibits K^+-evoked release of [^{3}H]-5-HT from preloaded slices of the rat frontal cortex (Middlemiss, 1984a). These effects of RU-24969 support the view that the 5-HT_1 receptor may be the autoreceptor involved in the regulation of 5-HT release and/or turnover (Martin & Sanders-Bush, 1982; Gozlan *et al.*, 1983).

In the present study we have examined the effects of RU-24969 on 5-HT release from the rat frontal cortex using both *in vitro* (superfusion) and *in vivo* (voltammetry and intracranial dialysis) techniques to identify further and establish a functional role for autoreceptor regulation of 5-HT release *in vivo*.

[1]Present address:- Department of Chemistry, Kansas University, Lawrence, Kansas 66045, U.S.A.
[2]Author for correspondence.

Methods

Superfusion of rat frontal cortex

Rats (230–260g) were killed by decapitation and the brains were placed on a frosted glass plate. The olfactory tracts were carefully separated from the inferior surface of the frontal cortex and a single coronal cut was made to separate the frontal cortex from the rest of the brain. Care was taken to ensure that no striatum was present in the frontal cortex preparation. The tissue weight range for each frontal cortex was 0.09–0.152g (mean 0.11g). The cortices from two rats were sliced (0.2 mm × 0.5 mm) with a fine wire grid slicing device (Bennett *et al.*, 1983). The pooled slices were incubated at 37°C for 20 min in 5 ml of freshly gassed Krebs solution. Composition mM: NaCl 118.5, KCl 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 1.3 and glucose 11.1 containing 10^{-7}M [^{3}H]-5-HT (specific activity 12.8 Ci $mmol^{-1}$, Amersham International PLC). Ascorbic acid (6×10^{-2}M) and disodium EDTA (3×10^{-2}M) were added to the incubation medium to prevent oxidation of the [^{3}H]-5-HT.

After washing, the slices were suspended in 1 ml of buffer and distributed between 8 superfusion cham-

bers which were superfused with gassed Krebs (37°C) containing 3×10^{-6}M paroxetine hydrochloride (Beecham pharmaceuticals Ltd) at a flow rate of 3–5 ml min^{-1}. The slices were superfused for an initial 10 min and then twenty 4 min fractions of the superfusate were collected. During 5th and 15th collection periods, 4 min pulses of Krebs containing 21 mM K^+ were introduced. Krebs containing either RU-24969 (Roussel) or 5-methoxy-N_1N-dimethyl tryptamine (5 MeODMT, Sigma Ltd) was introduced into only 4 of the 8 channels at the end of fraction 10 (i.e. after the first K^+ pulse, S_1), and in these 4 channels the second pulse of high K^+ Krebs solution (S_2) also contained the drug. The radioactivity in the slices and in each fraction was determined by liquid scintillation counting (Fisofluor 16 ml). Results are given as the % fractional release for the S_2 fraction in control and drug-treated slices.

In vivo *voltammetry and dialysis*

Electrode preparation Working electrodes, consisting of three carbon fibres (i.e. Carbone Lorraine, 8 μm o.d., ref: AGT/F), were prepared as described by Sharp *et al.* (1984) and Marsden *et al.* (1984). Electrical contact between the carbon fibres and the wire terminal of the electrode was achieved by injecting a carbon powder/epoxy resin mixture into the pulled glass capillary with a hypodermic needle and syringe. The electrode tip was sealed with epoxy resin by carefully placing a small drop of the epoxy on the capillary tip. After curing at 55°C for 1 h the fibres were cut to 0.5 mm and the electrode was electrically pretreated. This pretreatment, described by Sharp *et al.* (1984). sensitized the electrode to indoleamines. Reference and auxiliary electrodes were prepared as described previously (Sharp *et al.*, 1984).

Dialysis loop preparation The method adopted essentially followed that described by Ungerstedt (1984) and Sharp *et al.* (1984). Dialysis loops were prepared from flexible cellulose tubing (Gambro, 0.25 mm o.d.) with a molecular weight cut-off of 5000 Daltons. A 5 cm length of dialysis tubing was threaded through the lumen of two 1 cm cannulae, prepared from 23G stainless steel tubing. Both cannulae were glued to the dialysis tubing with rapidly setting epoxy resin, leaving 2 mm of dialysis tubing exposed between the two cannulae. To prevent the dialysis tubing from collapsing during manipulation and implantation, approximately 2 cm of very thin (0.1 mm o.d.), nylon fishing line was inserted in the lumen of the dialysis tubing, and positioned midway between the two cannulae. To facilitate perfusion, a 20°C angle bend was made at the end of one of the cannulae.

Just before implantation the angled cannula was connected via polythene tubing to a slow infusion pump (Scientific and Research Instruments). The dialysis tubing was perfused with Ringer solution (composition, M: NaCl 2.368, $NaHCO_3$ 0.5, KCl 0.475, $MgSO_4 7H_2O$ 0.118, KH_2PO_4 0.118 and $CaCl_2 2H_2O$ 0.252, pH 6.0 at 22°C) at a rate of 10 μl min^{-1}.

Once moistened, the dialysis tubing became flexible and was folded into a loop. The dialysis loop was continually perfused and was implanted vertically, using standard stereotaxic procedures (see below). During implantation additional support to the tubing was provided by a fine tungsten wire positioned outside the tubing. Perfusate (20 min samples) was collected from the cannula using a trimmed, inverted 'Eppendorf' tube containing 5 μl of perchloric acid (1 M) to reduce amine oxidation.

Animal preparation

Male Wistar rats (270–280g) were anaesthetized with chloral hydrate (600 mg kg^{-1}, i.p.) maintenance doses being given when required. Electrode and dialysis loop implantation followed standard stereotaxic procedures using a David Kopf frame with the upper incisor bar set + 5.0 mm above the interaural line. Two small holes (1 mm diam.) were drilled in the caudal part of the cranium and the reference and auxiliary electrodes positioned on the dura surface and held in place with chronoplastic dental acrylic. Further holes (2 mm diam.) were drilled above the left and right aspects of the pre-frontal cortex and the underlying dura membrane broken with a fine hypodermic needle, care being taken not to damage surface blood vessels. Co-ordinates for the pre-frontal cortex were measured from bregma and the dura surface according to Pellegrino *et al.* (1981) as follows: rostral-caudal + 3.6 mm; sagittal 2.0 mm; vertically − 2.0 mm.

A continually perfused dialysis loop was then implanted in the pre-frontal cortex and cemented in place. Following electrical pretreatment a carbon fibre electrode was tested and calibrated in a mixture of ascorbic acid (5×10^{-4}M) and 5-HIAA (5×10^{-5}M) by use of differential pulse voltammetry, and then carefully implanted in the contralateral pre-frontal cortex.

Parameters for voltammetry and dialysis

The voltammetric technique used, differential pulse voltammetry, involves the application of a steadily increasing ramp potential with superimposed regular step potentials to the implanted carbon fibre electrode. Current measurement is made for short periods (20 ms) immediately before a step (ia) and just before

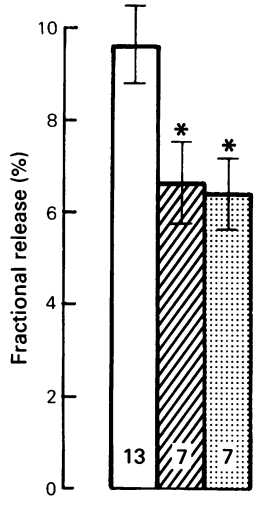

Figure 1 Effect of RU-24969 (1×10^{-5}M) (hatched column) and 5-methoxy-N,N-dimethyltryptamine (5×10^{-6}M) (stippled column) on K^+-stimulated [^{3}H]-5-HT release from slices of rat frontal cortex. Results are expressed as % fractional release for the S_2 fraction in control (open column) and drug-treated slices (mean values; vertical line shows s.e.). Numbers in the columns refer to the number of experiments. $*P < 0.05$ versus control value.

the end of a step (ib) and the current difference (ib − ia) plotted against the applied voltage.

Differential pulse voltammetry was performed (Princeton 174A polarograph) for both *in vitro* and *in vivo* studies with the following scan parameters: potential range −0.2 V to +0.45 V; scan rate 5 mV s^{-1}; modulation amplitude 50 mA; pulse frequency 2 s^{-1} with an *in vivo* scan frequency of one scan/5 min. Peak height (nA) was determined by drawing a line between the shoulders of the peak and measuring the perpendicular height between this line and the apex of the peak.

The dialysis loop was perfused at the rate of 1 μl min^{-1} and samples were collected every 20 min and analysed, without extraction, using h.p.l.c. with electrochemical detection for 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The amines and their metabolites were separated by reverse phase chromatography (Spherisorb 5 ODS 2, 25 cm × 5 mm), using a 0.1 M acetate-citrate buffer, pH 4.5, containing 10% methanol as the mobile phase. Electrochemical measurements were made using a carbon paste working electrode held at +0.65 V.

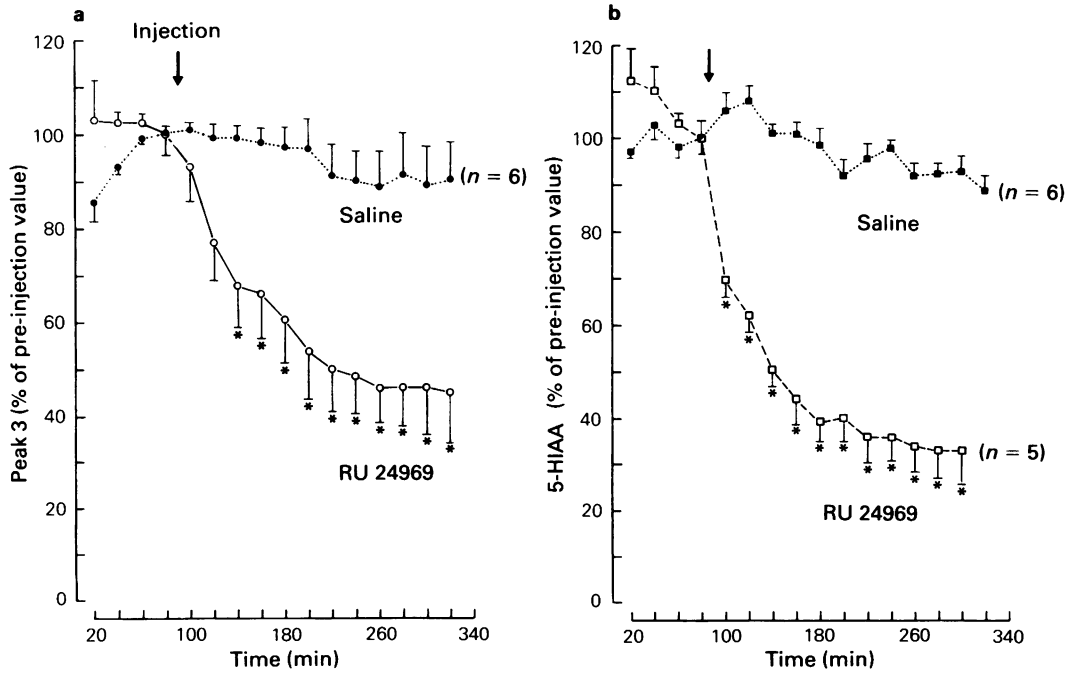

Figure 2 Effect of RU-24969 (10 mg kg^{-1}) (○) on extracellular 5-HIAA in the frontal cortex measured by voltammetry (a) and dialysis (b) in anaesthetized rats. Saline injected control animals (●). Injections (↓) were made 100 min after the start of the experiment. Note the similar decrease in 5-HIAA (dialysis) and peak 3 (voltammetry) following administration of RU-24969. $*P < 0.01$. Results are mean values with vertical lines showing s.e.mean.

Analysis of results

Voltammetric peak heights for each animal, initially recorded as nA, were finally expressed as a % of the baseline pre-injection control, a value calculated from the mean of the four peak heights measured immediately before drug administration. As there were four voltammetric readings to every dialysis sample, the voltammetric results obtained over the 20 min perfusion collection period are given as a mean value.

Dialysis results were also calculated as a % of the pre-injection control. The 100% value for the pre-injection control was taken as the height of the appropriate peak with reference to injected standards (10 pmol) on the h.p.l.c. trace for the 20 min sample collected immediately before drug administration.

Differences between drug and saline results were examined using Student's unpaired *t* test.

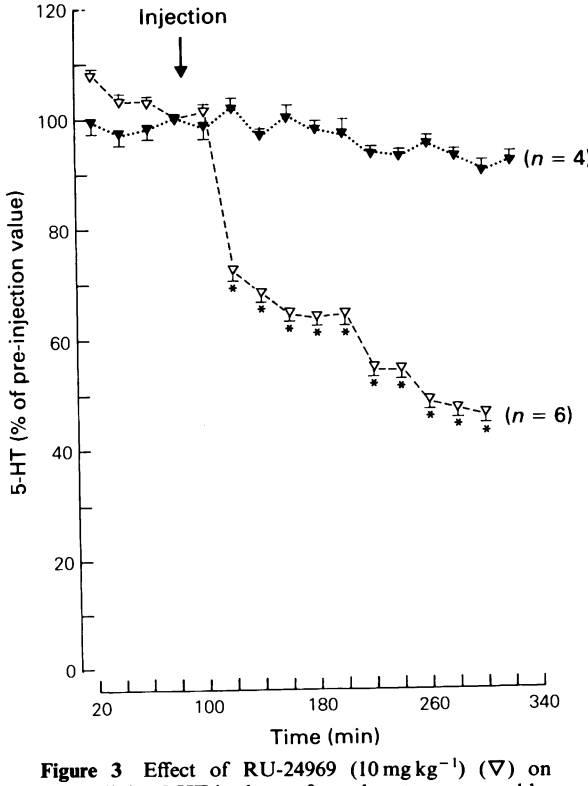

Figure 3 Effect of RU-24969 (10 mg kg^{-1}) (▽) on extracellular 5-HT in the rat frontal cortex measured by intracerebral dialysis in the anaesthetized rat, compared with saline injected control animals (▼). Injections (↓) were made 100 min after the start of the experiment. *$P < 0.01$, results given as mean value with vertical lines showing s.e.mean.

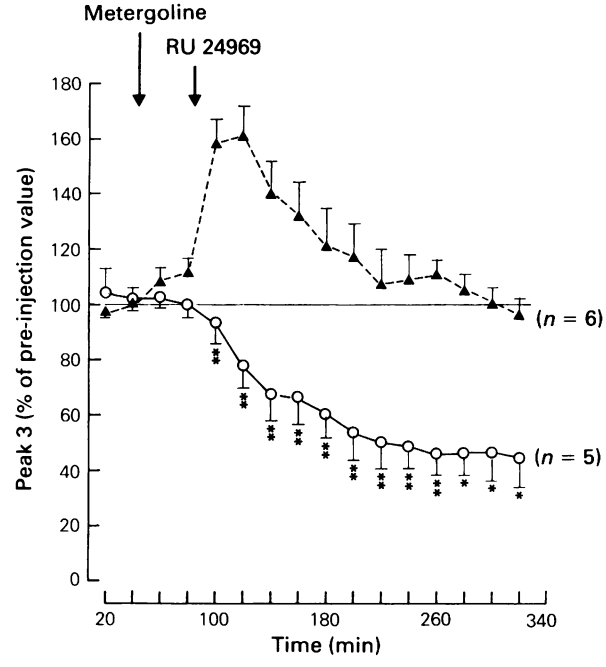

Figure 4 Effect of pretreatment with metergoline (2 mg kg^{-1}) (▲) on the effect of RU-24969 (10 mg kg^{-1}) (○) on peak 3 (5-HIAA) in the frontal cortex of the anaesthetized rat measured by voltammetry. Metergoline was administered 40 min before RU-24969. Results are expressed as mean with vertical lines showing s.e.mean. Note the increase in peak 3 in the presence of metergoline following administration of RU-24969 and the absence of the decrease seen with RU-24969 alone. *$P < 0.002$; **$P < 0.001$.

In vitro *capture of 5-HT and 5-HIAA by the dialysis loops*

To estimate the capture of 5-HT and 5-HIAA by the dialysis loops, these were perfused *in vitro* and placed in physiological saline containing 5-HIAA and 5-HT at 10^{-6}M. The amount of substance in the perfusate was compared with the amount outside the dialysis tube and expressed as % recovery. Recovery *in vitro* of 5-HT and 5-HIAA was $8.00 \pm 2.51\%$ and $7.75 \pm 2.4\%$ at a flow rate of 1.0 μl min^{-1}.

Drugs

The 5-HT$_1$ agonist 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole; (10 mg kg^{-1} i.p., RU-24969, Roussel), the non-selective 5-HT antagonist metergoline (2 mg kg^{-1} i.p., Farmitalia Carlo ERBA) and the selective 5-HT$_2$ antagonist ritanserin (R-55667) 1 mg kg^{-1} i.p., Janssen) were dissolved in 0.9% saline. Saline (2 mg kg^{-1}, i.p.) was used for control injections.

Results

Effects of RU-24969 and 5-MeODMT on K^+-stimulated release of [^{3}H]-5-HT from rat frontal cortex slices in vitro

K^+ (21 mM) increased fractional release of [^{3}H]-5-HT by between 120% and 150%. This increase was significantly reduced by both RU-24969 and 5-MeODMT (Figure 1). RU-24969 (1×10^{-5}M) decreased release by 30% while 5-MeODMT (5×10^{-6}M) caused a 33% reduction.

Effect of RU-24969 on 5-HT release and metabolism in vivo*: voltammetry and intracerebral dialysis*

Electrically pretreated carbon fibre electrodes gave two distinct oxidation peaks when tested *in vitro* in a mixture of ascorbic acid (5×10^{-4}M) and 5-HIAA (5×10^{-5}M). The first peak (at 0.0 V) was due to acorbic acid oxidation, and the second peak (+0.3 V) was to 5-HIAA oxidation. The height of this latter peak increase linearly in response to increasing 5-HIAA concentrations (5 to 100 μM).

On implantation in the pre-frontal cortex these electrodes produced two consistent oxidation peaks. The first, at approximately −0.075 V (peak 1), was due to the oxidation of ascorbic acid, while the second at +0.3 V (peak 3) was due primarily to the oxidation of 5-HIAA (Cespuglio *et al.*, 1981a,b; Sharp *et al.*, 1984).

For the *in vivo* dialysis results, the 100% pre-injection control values for extracellular 5-HIAA and 5-HT levels were 14.25 ± 0.4 (n = 5) and 1.03 ± 0.17 (n = 6) pmol 20 μl^{-1} respectively, after correction for recovery (see Methods).

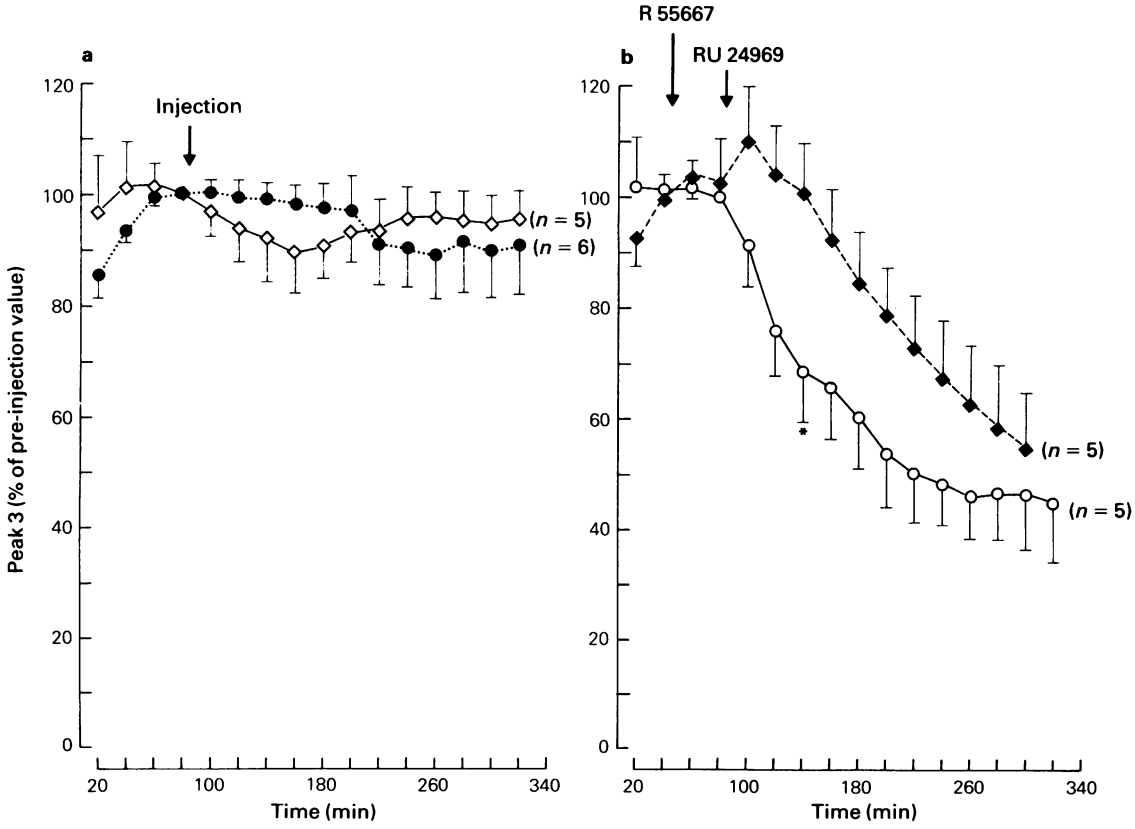

Figure 5 (a) Effect of ritanserin (R-55667, 1 mg kg^{-1}) (●) on peak 3 (5-HIAA) in the frontal cortex of the anaesthetized rat measured by voltammetry compared to saline-treated controls (○). Results are expressed as mean values with vertical lines showing s.e.mean. (b) Effect of pretreatment with R-44557 (1 mg kg^{-1}) (◆) on the effect of RU-24969 (10 mg kg^{-1}, ○) on peak 3 (5-HIAA) in the frontal cortex of the anaesthetized rat measured by voltammetry. R-55667 was administered 40 min before RU-24969. Results are expressed as mean values with vertical lines showing s.e.mean; *$P < 0.05$. Note that R-55667 alone has no effect on peak 3 while RU-24969 decreases peak 3 both in the absence and presence of R-55667.

Effect of RU-24969 on extracellular 5-HIAA and 5-HT in vivo

After RU-24969 (10 mg kg^{-1}, i.p.) administration, extracellular 5-HIAA, measured by simultaneous voltammetry and brain dialysis, fell rapidly and markedly (Figure 2). The decrease with both techniques followed approximately the same time course and % change.

With voltammetry the height of the 5-HIAA peak (peak 3) fell to 47% ($n = 5$) of the pre-injection control value within 240 min of RU-24969 administration with a significant difference between the saline and RU-24969 values from 60 min post injection (Figure 2a).

Similarly, with dialysis, extracellular 5-HIAA decreased to 33% ($n = 5$) of the pre-injection control, 240 min after RU-24969 administration with a significant difference between saline and RU-24979 values from 20 min post-injection (Figure 2b). This decrease in 5-HIAA was observed in both anaesthetized and freely moving animals.

Following RU-24969 (10 mg kg^{-1}) 5-HT, measured by dialysis, decreased to 45% ($n = 6$) of the pre-injection control value within 40 min of drug administration (Figure 3). This decrease, although of slower onset, followed a similar course to the decrease in 5-HIAA.

Effect of 5-HT antagonists on RU-24969-induced decrease in 5-HIAA in vivo

Following the administration of metergoline (2 mg kg^{-1}, i.p.), there was a small (<10%), transient but not significant increase in extracellular 5-HIAA. When RU-24969 was administered 40 min after metergoline the 5-HIAA oxidation peak increased initially to 60% above the pre-injection value (Figure 4). This change was significantly different from the decrease observed with RU-24969 alone or from the lack of effect with metergoline alone. Following this increase, extracellular 5-HIAA gradually decreased to the pre-injection value (Figure 4).

The selective 5-HT$_2$ antagonist, ritanserin (R-55667, 1 mg kg^{-1} i.p.), had no significant effect on the height of peak 3 within 80 min of drug injection (Figure 5a). Administration of RU-24969 to ritanserin pretreated animals caused an initial 10% increase in the electrochemical signal (Figure 5b) but the peak height then gradually fell and paralleled the decrease seen with RU-24969 alone.

Discussion

Intracerebral dialysis combined with h.p.l.c. and electrochemical detection provides positive identification of the substances passing into the perfusate through the dialysis membrane (Zetterstrom *et al.*, 1983; Sharp *et al.*, 1984; Ungerstedt, 1984). RU-24969 decreased the amount of 5-HT and 5-HIAA assayed in the perfusate. The decrease in extracellular 5-HIAA measured by intracerebral dialysis was paralleled by a decrease in the size of peak 3, the presumed 5-HIAA oxidation peak, monitored by differential pulse voltammetry using a carbon fibre electrode. Previous studies have demonstrated that inhibition of monoamine oxidase decreases the 5-HIAA measured by intracerebral dialysis and the size of peak 3 in a parallel manner (Sharp *et al.*, 1984), indicating that peak 3 represents 5-HIAA oxidation (Cespuglio *et al.*, 1984). It has also been demonstrated that up to 30% of peak 3 in the striatum could be accounted for by uric acid oxidation (Crespi *et al.*, 1983). The present study, however, indicates that changes in extracellular 5-HIAA closely follow changes in the height of peak 3.

The advantage of the dialysis method is the positive identification of the substances in the perfusate but its disadvantages include the size (1 mm diam.) of the probe and the length of time between each sample (20 min). Voltammetry offers an alternative method for measuring extracellular 5-HIAA, the advantages being the small size of the probe (20 μm tip diam.) and the short time interval between each recording (4 min). With voltammetry we have determined the *in vivo* pharmacological profile of the 5-HT receptor involved in the RU-24969-induced decrease in 5-HT release and metabolism.

A number of studies have indicated that RU-24969 has a potent and selective 5-HT$_1$ agonist action. For example, ligand binding studies have shown that RU-24969 has a high affinity for 5-HT$_1$ binding sites (Hunt & Oberlander, 1981). RU-24969 has been shown to decrease 5-HT turnover (Euvrard & Boissier, 1980) and decrease release of [^{3}H]-5-HT from rat frontal cortex (Middlemiss, 1984a), indicating that the 5-HT$_1$ receptor is the 5-HT autoregulator, regulating 5-HT release. This study confirms that RU-24969 decreases 5-HT release *in vitro* and demonstrates that the drug also decreases extracellular 5-HT and 5-HIAA *in vivo*, both in anaesthetized and freely moving rats. Evidence that the effects of RU-24969 are due to stimulation of 5-HT$_1$ receptors rather than 5-HT$_2$ receptors comes from the observation that the *in vivo* effects on extracellular 5-HIAA were prevented by pre-administration of the non-selective 5-HT antagonist, metergoline (Fuxe *et al.*, 1975; Gothert, 1980; Schlicker & Gothert, 1981), coupled with the observation that the 5-HT$_2$ selective antagonist R-55667, a substituted benzhydrylene (Laduron & Janssen, 1984) did not prevent the decrease in 5-HIAA. Metergoline itself produced a small but not significant increase in extracellular 5-HIAA *in vivo* in the frontal cortex which was similar to the increase observed in the

hippocampus *in vivo* (Baumann & Waldmeier, 1984). While various non-selective 5-HT antagonists enhance K^+-stimulated [^{3}H]-5-HT release *in vitro* (Engel *et al.*, 1983; Middlemiss, 1984b,c), further studies are required to determine whether the positive effects of 5-HT antagonists on 5-HT release *in vitro* are of functional importance *in vivo*. There is no clear explanation for the marked initial rise in extracellular 5-HIAA when RU-24969 was administered to rats pretreated with metergoline. It may suggest that RU-24969 has effects on a 5-HT receptor not blocked by metergoline, which is only observed when the other 5-HT receptor sub-types are antagonized. Alternatively the effect may be mediated by an effect of RU-24969 or metergoline on another neurotransmitter system.

In vivo voltammetry measures the extracellular levels of the metabolite only, and therefore there is a need to establish whether changes in 5-HIAA reflect changes in 5-HT release or changes in metabolism independent of release. In the present study the results with intracerebral dialysis indicate that extracellular 5-HIAA decreased before 5-HT, as 5-HIAA had fallen by 30% 20 min after administration of RU-24969 while no decrease was seen in 5-HT levels until 40 min post-injection. This could indicate that RU-24969 has other effects on 5-HT neurones apart from stimulating 5-HT$_1$ receptors, such as inhibiting 5-HT uptake, which would increase extracellular 5-HT while decreasing 5-HIAA, an effect that could initially mask the decrease in 5-HT release. Such results suggest that changes in 5-HT metabolism may precede changes in release and that under certain conditions extracellular 5-HIAA can decrease without a change in 5-HT release.

Using *in vivo* voltammetry and intracerebral dialysis we have established that RU-24969 decreases both metabolism and release of 5-HT *in vivo* and that the action on metabolism may precede that on release. The effects produced by RU-24969 support the view that 5-HT$_1$ autoreceptors are involved in the regulation of 5-HT release and metabolism.

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