Pharmacological characteristics of 5-hydroxytryptamine autoreceptors in rat brain slices incorporating the dorsal raphe or the suprachiasmatic nucleus

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1 Changes in extracellular concentrations of 5-hydroxytryptamine elicited by electrical stimulation in rat brain slices containing the dorsal raphe nucleus and the suprachiasmatic nucleus were monitored with fast cyclic voltammetry.

2 Using pseudo single pulse stimulation (5 pulses applied at 100 Hz) we have shown that the release of 5-hydroxytryptamine in the dorsal raphe and the suprachiasmatic nucleus can be regulated by autoreceptors in both brain regions.

3 In the suprachiasmatic nucleus, 5-carboxamidotryptamine, RU24969, 1-(*m*-trifluoromethylphenyl)piperazine and sumatriptan caused a concentration-dependent inhibition of stimulated 5-hydroxytryptamine overflow in the range 1×10^{-9} M to 3×10^{-6} M. The actions of 5-carboxamidotryptamine and RU24969 were reversed competitively by methiothepin (10^{-8} M to 10^{-6} M); Schild plots revealed pK_B values of 7.9 and 8.1. By contrast, ipsapirone and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) are not effective 5-hydroxytryptamine autoreceptor agonists in the suprachiasmatic nucleus.

4 Isamoltane (10^{-6} M), the putative 5-HT_{1B} receptor antagonist, blocked the responses to RU24969 (10^{-6} M) and 1-(*m*-trifluoromethylphenyl)piperazine (10^{-6} M) in the suprachiasmatic nucleus.

5 In the dorsal raphe nucleus, 8-OH-DPAT, ipsapirone, RU24969, 5-carboxamidotryptamine, and sumatriptan (all 1×10^{-8} M to 3×10^{-6} M) produced a concentration-dependent reduction in the stimulated release of 5-hydroxytryptamine. The maximum effect observed was less than that seen in the suprachiasmatic nucleus.

6 Methiothepin (1×10^{-7} M) blocked the effect of 5-carboxamidotryptamine (10^{-8} M to 10^{-6} M) in the dorsal raphe nucleus while propranolol (10^{-6} M) and NAN-190 (10^{-6} M) but not isamoltane (10^{-6} M) were found to block significantly the effect of ipsapirone (10^{-6} M).

7 We conclude, that drugs with 5-HT_{1A} binding activity act as agonists in the dorsal raphe nucleus while drugs showing some activity for 5-HT_{1B} and 5-HT_{1D} binding sites, act as agonists in the suprachiasmatic nucleus. Our results confirm predictions from binding studies, that functional 5-HT autoreceptors regulating release of endogenous 5-HT have different drug specificity in the dorsal raphe and suprachiasmatic nucleus.

Keywords: 5-HT autoreceptors; 5-HT_{1A} agonists; 5-HT_{1B} agonists; methiothepin; dorsal raphe nucleus; suprachiasmatic nucleus; fast cyclic voltammetry

Introduction

Ligand binding studies *in vitro*, and autoradiographic studies in brain slices, indicate a heterogeneity of 5-hydroxytryptamine (5-HT) receptors in the rat brain (Peroutka & Snyder, 1979; Bradley *et al.*, 1986; Hoyer *et al.*, 1986a,b; Peroutka, 1988a,b). Three types of 5-HT receptor (designated 5-HT_{1,2,3}) have been described on the basis of work with ligands, which have subsequently been shown to be either agonists or competitive antagonists of 5-HT receptors in functional studies (see Bradley *et al.*, 1986; and Peroutka, 1988a for reviews); there is speculation about the existence of 5-HT₄ receptors (Schenker *et al.*, 1987; Dumuis *et al.*, 1989).

5-HT₁ receptors were originally characterized as having selective affinity for 5-HT, and differentiated from 5-HT₂ receptors which had selective affinity for spiperone (Peroutka & Snyder, 1979). Subsequent studies divided 5-HT₁ receptors into four subtypes, based on affinity of agonists, brain distribution and species. 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (Middlemiss & Fozard, 1983) and ipsapirone (Domper *et al.*, 1985) were shown to be selective agonists at 5-HT_{1A} receptors. They were found postsynaptically in cortex

of all species examined (Hoyer *et al.*, 1986a), and presynaptically in the dorsal raphe nucleus of the rat (De Montigny *et al.*, 1984; Sprouse & Aghajanian, 1986) where they serve as 5-HT autoreceptors. It has been suggested (Sills *et al.*, 1984) that RU24969, and TFMPP are selective ligands for 5-HT_{1B} receptors on 5-HT axon terminals in the rat (Pazos & Palacios, 1985), and that 5-HT_{1B} receptors serve as autoreceptors at these sites (Middlemiss, 1984; Engel *et al.*, 1986). In species other than rat (including man, calf, pig and guinea-pig), it has been generally accepted that axon terminal 5-HT autoreceptors constitute a separate class designated 5-HT_{1D} (Heuring & Peroutka, 1987; Waeber *et al.*, 1988), although Herrick-Davis & Titeler (1988) and Limberger *et al.* (1991) suggest that both 5-HT_{1B} and 5-HT_{1D} receptors may coexist in the rat. Sumatriptan (GR 43175; Humphrey *et al.*, 1988) has been shown to be a selective agonist at 5-HT_{1D} receptors. 5-HT_{1C} receptors have been found in all mammalian species examined, present on the choroid plexus only (Pazos *et al.*, 1984; Hoyer *et al.*, 1989); they have not been associated with 5-HT autoreceptor function.

No selective competitive antagonists have been described for 5-HT₁ receptor subtypes (Peroutka, 1988a,b). Methiothepin can competitively block all 5-HT₁ receptor subtypes as well as 5-HT₂ receptors; propranolol has antagonistic actions at 5-HT_{1A} and 5-HT_{1B} receptor types as well as at β -adrenoceptors (Göthert, 1990). NAN-190 has antagonistic activity at

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5-HT_{1A} receptor subtypes (Glennon *et al.*, 1988) and isamoltane (Schoeffter & Hoyer, 1989) has weak selectivity for 5-HT_{1B} receptors.

Among the mechanisms regulating 5-HT release at both somatodendritic and axon terminal sites, are 5-HT autoreceptors (reviewed by Starke *et al.*, 1989; Kalsner & Westfall, 1990). Electrophysiological and behavioural studies *in vivo*, have shown that 5-HT_{1A} receptor agonists applied to the dorsal raphe nucleus, act on 5-HT autoreceptors which regulate firing of 5-HT neurones (De Montigny *et al.*, 1984; Dourish *et al.*, 1986; Vander Maelen *et al.*, 1986; Sprouse & Aghajanian, 1986; Haj-Dahmane *et al.*, 1991). The consequences of changes in the electrical activity of 5-HT nerve cells on release of 5-HT at axon terminals has been investigated *in vivo* by voltammetry and dialysis. When 5-HT_{1A} selective agonists were applied either systemically or into the dorsal raphe nucleus (Marsden & Martin, 1986; Sharp *et al.*, 1989) activation of 5-HT_{1A} receptors in the raphe nuclei lead to decreased 5-HT metabolism and release at axon terminals. At 5-HT axon terminals, 5-HT_{1B} agonists have been shown to decrease 5-HT release and metabolism by acting at terminal 5-HT autoreceptors (Middlemiss, 1985; Martin & Marsden, 1986; Limberger *et al.*, 1991).

The involvement of 5-HT in many neurological, psychiatric and neuroendocrine disorders means that there is a real prospect of novel therapies for a wide range of clinical disorders, if drugs acting selectively and specifically at 5-HT receptors can be developed (Meltzer, 1990). Discovery of such agents depends on qualitative and quantitative characterization of 5-HT receptors in the central nervous system. While electrophysiological studies have good anatomical precision, quantitative characterization of the receptors involved can be difficult *in vivo*, as the concentration of drugs can be difficult to measure. By contrast, in biochemical studies *in vitro* where the concentration of ligands can readily be determined, the site of action of the drug may be difficult to determine in either brain slices or broken cell preparations.

We have shown that fast cyclic voltammetry can monitor changes in the extracellular concentration of 5-HT following electrical stimulation in either the dorsal raphe nucleus (DRN) or the suprachiasmatic nucleus (SCN; O'Connor & Kruk, 1991a,b). This method is anatomically precise, as the electrodes are small (being an exposed carbon cylinder measuring 7 µm by 30 µm). Quantitative pharmacological characterization of dopamine autoreceptors is possible, when fast cyclic voltammetry is applied in suitably prepared brain slices (Bull *et al.*, 1990; Bull & Sheehan, 1991; Palij *et al.*, 1990). In order to study pulse to pulse regulation of release of 5-HT and to measure effects of agonists at 5-HT autoreceptors, it is necessary to work under conditions when the endogenously released 5-HT does not act to modulate its own release (Singer, 1988). We have shown (O'Connor & Kruk, 1991b) that single pulse electrical stimulation does not release sufficient 5-HT for it to be measurable reliably above the background. To overcome this, we have used a short burst of stimuli (pseudo single pulse stimulation; Singer, 1988; Cejna *et al.*, 1990; Limberger *et al.*, 1991) which we have shown causes 5-HT release which is unaffected by the presence of methiothepin, indicating that such short periods of stimulation do not activate 5-HT autoreceptors.

Here, we describe results of qualitative and quantitative experiments in which we have studied the effects of 5-HT₁ ligands on their ability to modify release of endogenous 5-HT in an axon terminal region (SCN) or at a somatodendritic site (DRN). A preliminary account of some of these findings has been given previously (O'Connor & Kruk, 1990; 1991c).

Methods

All experiments were carried out on rat brain slices prepared and treated as has been described in detail previously

(O'Connor & Kruk, 1991a); only a brief outline is presented here.

Preparation of brain slices

Male Wistar rats (150–250 g) were killed and the brain gently and rapidly removed into ice cold oxygenated artificial cerebrospinal fluid (ACSF; composition in mM: NaCl 125, KCl 2.0, KH₂PO₄ 1.25, MgSO₄·7H₂O 2.0, NaHCO₃ 25.0, D-glucose 11.0, CaCl₂ 2.0) and saturated with 5% CO₂ in oxygen. Blocks of tissue, 3 mm thick containing either DRN or SCN were dissected with a razor blade, and a single slice (350 µm) containing either DRN or SCN was prepared with an Oxford vibratome. The slice was transferred to a full immersion incubation chamber (Richards & Tegg, 1977) perfused at a rate of 1.1 ml min⁻¹ with artificial oxygenated ACSF maintained at 32°C, and allowed to equilibrate for 1 h.

Fast cyclic voltammetry

Following a 1 h equilibration period, a single carbon fibre microelectrode (8 µm diameter and 30–50 µm exposed tip) was located 80 µm below the surface of the brain slice in either brain region, and a bipolar stimulating tungsten microelectrode (tip separation 200 µm) was implanted at the same depth about 100–200 µm away from the carbon fibre electrode. Fast cyclic voltammetry was applied twice a second, and the oxidation peak of 5-HT (+600 mV) was monitored with a sample and hold device, and displayed on a chart recorder. Variations in the height of the peak at +600 mV were used to monitor changes in the extracellular concentration of 5-HT during electrical stimulation. A triphasic W shaped voltage wave form was applied, and the applied potential as well as the resulting current waveform were monitored on a digital storage oscilloscope (Nicolet 310; Figure 1a and b). Fast cyclic voltammetry was performed with a Millar voltammeter (PD Systems, West Molsley, Surrey) and voltammetric current signals were applied to a CED 1401 interface for digitalization and storage of wave forms on hard disk using CED signal averaging (Sigavg) software. Electrodes were calibrated in solutions of 5-HT before and after each experiment; concentrations in brain slices were determined by interpolation of calibration curves (see O'Connor & Kruk, 1991a).

Electrical stimulation

In the DRN or SCN single pulse (0.1 ms width, 20 V amplitude; 1P), 5 single pulses applied at 100 Hz (5P/100 Hz) or repeated stimulation was controlled by a Neurolog system incorporating a DS2 constant voltage stimulator.

Experimental procedure

All drugs were dissolved in ACSF and applied at increasing concentration in a cumulative manner. Each agonist concentration was superfused until the response achieved a plateau (less than 30 min). In the studies using methiothepin and 5-CT or RU24969, a concentration-response curve was carried out with the agonist and the slice was then washed with drug free ACSF. The slice was then equilibrated with a single concentration of methiothepin (10⁻⁸, 10⁻⁷ or 10⁻⁶ M) for 1 h before a second agonist concentration-response curve was performed. Concentration-ratios were calculated at the EC₅₀ level and used to construct Schild plots (Arunlakshana & Schild, 1959; Kennakin, 1984) in order to derive pK_B values. The maximum % inhibition of 5-HT overflow (E_{max}) caused by each agonist was calculated. On occasion (for example see Figure 6) a single concentration (10⁻⁶ M) of agonist was perfused into the slice chamber for 30 min. The slice was then equilibrated with antagonist alone for 1 h before the same concentration of agonist was perfused in the presence

of the antagonist. Maximum changes in stimulated 5-HT overflow were then calculated for the agonist in the absence and in the presence of the antagonist.

Drugs and solutions

Drugs were obtained from the following sources: 5-hydroxytryptamine (5-HT) HCl (Sigma); TFMPP (1-(*m*-trifluoromethylphenyl)piperazine; Research Biochemicals Incorporated), 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin; Research Biochemicals Incorporated). The following were generous gifts from the sources indicated: methiothepin maleate (Hofmann La Roche), RU24969 (5-methoxy-3(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole; Russel Uclaf), ipsapirone (TVX Q 7821; Troponwerke, Koln, Germany), NAN-190 (1-(2-methoxyphenyl)-4-[4-2 (phthalimido) butyl]-8-azaspiro (4,5) decane-7,9-dione; Dr Richard Glennon, Medical College of Virginia, Richmond, VA, U.S.A.), isamoltane (Ciba Geigy) and buspirone HCl (Bristol-Myers Company). We thank Glaxo Group Research for the following drugs: 5-carboxamidotryptamine (5-CT) and sumatriptan (GR43175; 3-(2-dimethylamino)ethyl-N-methyl-1H-5-methane sulphonamide).

Statistical analysis

Examples of raw data are shown where appropriate. Means and standard deviations were determined from pooled data and levels of statistical significance were determined by paired or unpaired Student's *t* test as appropriate. Slopes and intercepts in Schild plots were determined from linear regression programmes available on an Apple Macintosh computer (Cricket Graph and Statsview 512).

Results

Voltammetric identification of release substance

The applied potential waveform and the charging current flowing in the carbon fibre microelectrode are shown in Figure 1a and b respectively. The upper trace in Figure 1b represents the faradaic current superimposed on the charging current obtained in the SCN of the rat during a train of 50 pulses applied at 100 Hz. Digital subtraction of the charging current (oscilloscope or computer) reveals the faradaic current (Figure 1c). A single oxidation peak (O) is seen at +600 mV and two reduction peaks (*r*₁ and *r*₂) are observed at 0 and -200 mV respectively. Figure 1d shows the faradaic current obtained in the DRN during a train of 25 pulses applied at 100 Hz. Figure 1e shows the faradaic current in the presence of 1×10^{-7} M 5-HT *in vitro*.

Effect of 5-HT receptor agonists on stimulated 5-HT overflow evoked by 25 pulses in the DRN and SCN

Figure 2 shows the results from a typical experiment in which changes in stimulated 5-HT overflow evoked by trains of 25 pulses were monitored for about 2 h in either the DRN (Figure 2a) or the SCN (Figure 2b) in the presence of 5-CT. A concentration-dependent decrease in stimulated 5-HT overflow was seen and a maximal response of 64% inhibition was observed in the SCN. In a series of studies summarized in Figure 3, the effects of 5-CT, buspirone, 8-OH-DPAT and RU24969 were shown to decrease electrically stimulated 5-HT overflow in the DRN (Figure 3a) and SCN (Figure 3b) in a concentration-dependent manner. EC₅₀ values and maximum inhibition of release (*E*_{max}) in both regions are summarized in Table 1 (columns 1/2 and 5/6). Evidence from experiments using low concentrations of methiothepin (results not shown; for full details see O'Connor & Kruk, 1991b) indicated that 25 pulses at 50 Hz released endogenous 5-HT which could activate 5-HT autoreceptors in both brain regions, 5 pulse stimulation (see Introduction) was used in all

subsequent experiments to avoid this potentially confounding effect.

Effects of 5-HT₁ receptor agonists on changes in stimulated 5-HT overflow evoked by 5 pulse stimulation in the SCN

Five pulses at 100 Hz stimulation applied once every 5 min for up to 6 h in either DRN or SCN resulted in reproducible

Figure 1 Voltammetric driving ramp and currents. The bar at the foot of the figure denotes time and applies to all traces. (a) Driving voltage ramp applied to the carbon fibre electrode. (b) Charging and charging plus faradaic currents flowing in the carbon fibre electrode in a rat brain slice containing the suprachiasmatic nucleus (SCN) before and following a 50 pulse/100 Hz stimulation. (c) Faradaic current produced after digital subtraction of the charging currents (shown in b) following the 50 pulse/100 Hz stimulation in the SCN. (d) Faradaic current produced after digital subtraction of the charging currents following a 25 pulse/100 Hz stimulation in the dorsal raphe nucleus (DRN). (e) Faradaic current produced due to the presence of $0.1 \mu\text{M}$ 5-hydroxytryptamine (5-HT), following digital subtraction of the charging current. The peak oxidation and reduction potentials are indistinguishable in (c), (d) and (e).

Figure 2 Sample and hold output traces showing the effects of 5-carboxamidotryptamine (5-CT) at the concentrations indicated upon the height of the peak 5-hydroxytryptamine (5-HT) oxidation signal (+600 mV). Electrical stimulation was applied once every 5 min in either (a) the suprachiasmatic nucleus or (b) dorsal raphe nucleus. After experiments the electrodes were calibrated in exogenous 5-HT at the concentrations indicated by the scale bar.

Table 1 Effect of 5-HT₁ receptor ligands on stimulated 5-hydroxytryptamine (5-HT) overflow evoked by trains of 25 pulses delivered at 50 Hz (25P/50 Hz) and trains of 5 pulses delivered at 100 Hz (5P/100 Hz)

Agonist	DRN				SCN			
	25P/50 Hz		5P/100 Hz		25P/50 Hz		5P/100 Hz	
	EC ₅₀	E _{max}	EC ₅₀	E _{max}	EC ₅₀	E _{max}	EC ₅₀	E _{max}
5-CT	21 ± 3	47.2 ± 7.8	25 ± 11	48 ± 7	67 ± 16	64.4 ± 6.5	5.6 ± 3.1	86 ± 6
RU24969	39 ± 19	29 ± 5.8	23 ± 13	51 ± 7.5	14 ± 2	50.7 ± 6	3.8(2.5)	89 ± 5
8-OH-DPAT	25 ± 6	44.2 ± 4.3	35 ± 19	43 ± 9	36 ± 18	24.2 ± 3.2	—	12 ± 4
Buspirone	65 ± 35	42.7 ± 7.8	NC	NC	140 ± 85	24 ± 9.5	NC	NC
Ipsapirone	NC	NC	12 ± 10	50 ± 6	NC	NC	—	-4 ± 5
Sumatriptan	NC	NC	180 ± 80	36 ± 8	NC	NC	42 ± 29	70 ± 6
TFMPP	NC	NC	NC	NC	NC	NC	5.0 ± 3.4	90 ± 5

EC₅₀: concentration of agonist (nM) required to reduce 5-HT to 50% control values. E_{max}: maximum inhibitory effect (%) of agonists. NC: experiments not carried out. *n* = 3–7 for each value (± s.e.mean). For abbreviations, see text.

and stable release of 5-HT (O'Connor & Kruk, 1991b). The effects of RU24969 at 3 different concentrations on 5 pulse stimulated 5-HT overflow in the SCN are shown in Figure 4a. Pooled results for RU24969, TFMPP, 8-OH-DPAT, ipsapirone, sumatriptan, and 5-CT are shown in Figure 4b. In the SCN, RU24969, 5-CT, and sumatriptan (3×10^{-9} to 1×10^{-6} M) caused a concentration-dependent decrease in 5 pulse stimulated 5-HT overflow. By contrast, ipsapirone and 8-OH-DPAT did not decrease stimulated 5-HT overflow significantly following 5 pulse stimulation.

The order of potency of the agonists, and comparison of the concentration needed to produce a 50% decrease in the maximum response, and the maximum effect obtainable with each agonist in both brain regions is summarized in Table 1 (columns 7 and 8).

Effect of methiothepin and isamoltane on the response to 5-HT₁ receptor agonist in the SCN

Addition of the non selective 5-HT₁/5-HT₂ receptor antagonist, methiothepin, had no significant effect on 5 pulse stimulation on its own (see above). However, methiothepin caused a concentration-dependent parallel shift to the right of the concentration-response curves to RU24969, 5-CT or

sumatriptan in the SCN (Figure 5a,b and c, respectively). The same maximum response was obtained to bath-applied RU24969, 5-CT and sumatriptan in the presence of the antagonist. In Figures 5d and 5e, Schild plots are drawn for RU24969 and 5-CT respectively. Simple linear regression analysis showed that the slope in each case was not significantly different from unity (0.83 and 1.18 respectively), and the pK_B for methiothepin with RU24969 was 7.9 and with 5-CT was 8.1.

Isamoltane (10^{-6} M), the putative 5-HT_{1B} receptor antagonist was found to attenuate significantly the maximum response to RU24969 (10^{-6} M; $P < 0.01$; $n = 4$, compared to controls) and TFMPP (10^{-6} M; $P < 0.01$; $n = 4-5$, compared to controls) in the SCN (Figure 6a and b).

Effects of 5-HT₁ receptor agonists on stimulated 5-HT overflow evoked by 5 pulse stimulation in the DRN

The effects of 8-OH-DPAT at two different concentrations on 5 pulse stimulated 5-HT overflow in the DRN is shown in Figure 7a. Ipsapirone, RU24969, 5-CT and sumatriptan decreased 5-HT release in a concentration-dependent manner in response to 5 pulse stimulation in the concentration-range 10^{-9} M to 10^{-6} M (Figure 7b). These compounds proved to

be less potent in the DRN than in the SCN, and even when the concentration of agonists was increased above 10^{-6} M in no case was it possible to obtain a complete inhibition of 5-HT release. Table 1 (columns 3 and 4) summarizes the ED_{50} and maximum effect of each agonist on 5 pulse stimulated 5-HT overflow in the DRN.

Figure 3 The effects of 5-HT₁ receptor ligands on stimulated 5-hydroxytryptamine overflow ([5-HT]ex) evoked by trains of 25 pulse/50 Hz in (a) the dorsal raphe nucleus or (b) the suprachiasmatic nucleus. The effects of 5-carboxamidotryptamine (●), RU24969 (□), buspirone (○) and 8-hydroxy-2-(di-n-propylamino)tetralin (■) at the concentrations indicated are shown for both regions. Results are expressed as percentage inhibition of release when compared to predrug controls (mean with s.e.mean shown by vertical bars; $n = 4-5$) at each observation point. This figure is derived from data such as that shown in Figure 2 for a single experiment.

Figure 4 The effects of 5-HT₁ receptor ligands on stimulated 5-hydroxytryptamine overflow ([5-HT]ex) evoked by 5 pulse 100 Hz stimulation in the suprachiasmatic nucleus (SCN). (a) Sample and hold output, set at the peak oxidation potential for 5-HT (+600 mV), for a single experiment during application of RU24969 at the concentrations indicated. Vertical bar: 10 nM 5-HT; horizontal bar: 10 s. (b) The effects of 5-carboxamidotryptamine (●), RU24969 (□), sumatriptan (▲), TFMPP (○), ipsapirone (■) and 8-hydroxy-2-(di-n-propylamino)tetralin (△) on electrically evoked endogenous 5-HT overflow in brain slices containing the SCN. The drugs were applied at the concentrations indicated and percentage inhibition was calculated by comparison in each case with pre-drug control release. Electrical stimulation consisted of 5×0.1 ms pulses; 100 Hz; 20 V; applied every 5 min. Each point shows the mean with s.e.mean indicated by vertical bars; $n = 4-5$.

Effect of 5-HT receptor antagonists on the response to 5-HT₁ receptor agonists in the DRN

Figure 7c shows the concentration-response curve for 5-CT in the absence and presence of methiothepin in the DRN. There was a significant reduction in the inhibition of stimulated 5-HT overflow produced by 5-CT in the presence of methiothepin ($P < 0.05$ for 10^{-7} M and 10^{-6} M 5-CT; $n = 4-5$).

Isamoltane the putative 5-HT_{1B} receptor antagonist did not significantly attenuate the response to ipsapirone in the DRN (results not shown; $P < 0.73$; $n = 4-5$, maximum inhibition in controls versus maximum inhibition in the presence of isamoltane).

NAN-190 (1×10^{-6} M) the putative 5-HT_{1A} antagonist when perfused in the presence of ipsapirone (10^{-6} M) significantly antagonized its effect in the DRN (results not shown; $P < 0.01$; $n = 4$; compared to controls).

Propranolol in the range 1×10^{-8} M to 1×10^{-5} M had no effect on stimulated 5-HT overflow in the DRN on its own ($n = 4$). However, the response to ipsapirone (10^{-6} M) was attenuated after pretreatment with 10^{-6} M propranolol (results not shown; $P < 0.5$; $n = 4$, compared to controls).

Figure 5 The effects of methiothepin applied at different concentrations on the concentration-response curves to (a) RU24969, (b) 5-carboxyamidotryptamine (5-CT) and (c) sumatriptan in the suprachiasmatic nucleus. Agonists were applied at the concentrations indicated either on their own ($\square$) or in the presence of 0.1 μ M methiothepin ($\circ$) or 1.0 μ M methiothepin (Δ). Each point shows the mean with s.e. mean indicated by vertical bars; $n = 4-5$. (d) and (e) show the Schild plots derived from the data shown in (a) and (b) respectively. The slopes of the calculated regression line did not differ from unity in either case (0.81 (d) and 1.2 (e)). The effect of 0.01 μ M methiothepin on the concentration-response curves to RU24969 and 5-CT is not shown in (a) or (b) for clarity.

Discussion

The use of a brain slice (where axon terminals and somatodendritic sites are separated), is advantageous for receptor characterization studies. While the use of brain slices had its limitations in that afferent inputs may be removed, for pharmacological experiments where the characteristics of autoreceptors are studied both qualitatively and quantitatively, this can be regarded as an advantage. In this study, we were able to study the pharmacology of the axon terminal autoreceptor in the absence of influences from the nerve cell body. The suprachiasmatic nucleus was chosen as a representative axon terminal site, as it possesses one of the highest concentrations of 5-HT located in axon terminals in the brain (Saavedra *et al.*, 1974b). The dorsal raphe nucleus was used as a representative somatodendritic structure which has been extensively studied.

In vivo voltammetric studies in which either 5-hydroxyindoleacetic acid was used as an index of 5-HT release (Marsden & Martin 1985; 1986) or measurement of endogenous 5-HT in dialysates (Sharp *et al.*, 1990) have produced qualitative information about 5-HT receptor subtypes, but such studies do not allow quantitative characterization of autoreceptors at either nerve cell bodies or axon terminals. Systemic administration of drugs in these studies makes it difficult to identify a particular anatomical target for action, although local application of the drug helps. When drugs are applied systemically, the influence of changes in the firing

rate of 5-HT nerve cell bodies makes interpretation of the measurement of 5-HT concentrations at axon terminals difficult, especially if an inhibitor of 5-HT uptake is routinely added to the dialysis fluid (Sharp *et al.*, 1989; 1990).

We have shown in other studies (O'Connor & Kruk, 1991a,b) that fast cyclic voltammetry can be used to measure rapid (less than 500 ms) changes in the extracellular concentration of 5-HT in the dorsal raphe nucleus or in the suprachiasmatic nucleus of the rat. In the present study we have shown that the voltammetric signal observed following electrical stimulation is indistinguishable from the signal obtained with exogenous 5-HT. In contrast to dopamine systems, we have found that it is not possible to obtain reproducible release of 5-HT following single pulse stimulation. Bull *et al.* (1990) were able to demonstrate dopamine release in response to single electrical pulse stimulation in the caudate putamen and nucleus accumbens; this may reflect the higher concentration of dopamine found at these sites. Our inability to demonstrate 5-HT release following single pulse stimulation may reflect relatively lower concentrations of 5-HT in the DRN and SCN (Saavedra *et al.*, 1974a,b). Five pulse stimulation at 100 Hz (a stimulus resulting in release of endogenous transmitter sufficiently brief to avoid activation of autoreceptors) has been shown to be an effective tool to study regulation of [3 H]-noradrenaline (Valenta *et al.*, 1988; Singer, 1988), [3 H]-dopamine (Mayer *et al.*, 1988), endogenous dopamine (Trout & Kruk, 1991) and [3 H]-5-HT (Singer, 1988; Fischer *et al.*, 1990; Limberger *et al.*, 1990; 1991).

Figure 6 The effects of 5-hydroxytryptamine (5-HT) receptor antagonists on stimulated 5-HT overflow ([5-HT]ex) in the suprachiasmatic nucleus (SCN). (a) The time course of effect of isamoltane (1 μM) on inhibition of 5 pulse at 100 Hz stimulated endogenous 5-HT overflow by TFMPP (1 μM) in slices containing the SCN. Each point shows the mean with s.e.mean indicated by vertical bars; *n* = 4. The open bar shows the time course while TFMPP was perfused; the black bar shows the time course of perfusion of isamoltane. (b) The effects of isamoltane (1 μM) on RU24969 (1 μM)-induced inhibition of 5-HT overflow is shown in the form of histograms. Each column shows the mean (s.e.mean indicated by vertical bars) (*n* = 4; **P* < 0.01; Isa: isamoltane; RU: RU24969).

Initially we examined the ability of 5-HT receptor agonists to inhibit endogenous 5-HT release elicited by trains of 25 pulses applied at 50 Hz (500 ms duration of stimulation). While 5-CT and RU24969 were more potent than ipsapirone or 8-OH-DPAT at inhibiting endogenous 5-HT release in the SCN, the same four agonists were approximately equipotent when their ability to inhibit release of 5-HT was examined in the DRN. In neither region did any of the drugs cause a full inhibition of stimulated 5-HT overflow. Experiments using methiothepin (0.1 μM; O'Connor & Kruk, 1991b) indicated that stimulations with 25 pulses at 50 Hz released endogenous 5-HT which activated 5-HT autoreceptors in both brain regions. It is likely that the perfused exogenous agonist competes with the released endogenous 5-HT when these stimulation parameters are used. This may account for the high EC₅₀ values observed in the SCN when compared to the EC₅₀ values obtained with stimulations of 5 pulses at 100 Hz (Table 1 columns 5 and 7).

Five pulse stimulation was used in all subsequent experiments to avoid the potentially confounding effects of the endogenous 5-HT inhibiting its own release or preventing

Figure 7 The effect of 5-HT₁ receptor ligands on stimulated 5-hydroxytryptamine overflow ([5-HT]ex) evoked by 5 pulses at 100 Hz in the dorsal raphe nucleus (DRN). (a) Sample and hold outputs showing traces from a single experiment in which 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) was applied at the concentrations indicated. Horizontal bar: 10 S; vertical bar: 10 nm 5-HT. (b) The effects of 5-HT₁ ligands present at the concentrations indicated upon inhibition of overflow of endogenous 5-HT evoked by 5 pulses at 100 Hz in brain slices containing the DRN (8-OH-DPAT (■), 5-carboxamidotryptamine (5-CT, ○), ipsapirone (Δ), sumatriptan (▲) and RU24969 (□); each point shows the mean (s.e.mean shown by vertical bars; *n* = 4–7). (c) The effect of 0.1 μM methiothepin (●) on the concentration-response curve to 5-CT (○) in the DRN; each point shows the mean with s.e.mean indicated by vertical bars; *n* = 4–7.

access of exogenously applied drug to the autoreceptors under study. Using this stimulus, we have shown (O'Connor & Kruk, 1991b) that release of endogenous 5-HT is unaffected by the presence of 1 μM methiothepin, thus indicating that this stimulus is not susceptible to autoreceptor regulation. Therefore by using 5 pulses at 100 Hz stimulation in both regions, we were able to study the effects of agonists and antagonists in the absence of autoinhibition by endogenous 5-HT.

Using the agonists and 5 pulses at 100 Hz stimulation, it was possible to establish an agonist potency series in the SCN. RU24969, TFMPP and 5-CT were virtually equipotent, more potent than sumatriptan, much more potent than 8-OH-DPAT and ipsapirone. The order of potency of agonists in this study is in accord with that described by

Limberger *et al.* (1991) who measured the ability of 8-OH-DPAT, 5-CT and RU24969 to inhibit [3 H]-5-HT release in slices of rat cortex. It is interesting to note, that in common with Limberger *et al.* (1991), we find that perfused exogenous 5-HT (in the presence of 1×10^{-6} M fluvoxamine to block 5-HT uptake) inhibited in a concentration-dependent manner, 5 pulse stimulated 5-HT overflow in the SCN, and had a potency intermediate between that of RU24969, 5-CT, TFMPP and 8-OH-DPAT (EC_{50} 110 nM; E_{max} 82%; O'Connor & Kruk, unpublished observations). 5-HT applied to the perfusion medium in the absence of uptake inhibitor had very little effect on stimulated 5-HT overflow, even when concentrations as high as 1μ M 5-HT were used.

None of the agonists studied caused complete inhibition of stimulated 5-HT overflow in the dorsal raphe nucleus. This does not imply that these drugs were acting as partial agonists, since 5-HT (in the presence of fluvoxamine; 1μ M) caused a similar degree of inhibition as that exerted by the more selective agonists used (O'Connor & Kruk, unpublished observations). We conclude that either a full agonist for 5-HT $_A$ autoreceptors has yet to be discovered, or electrically stimulated 5-HT release cannot be completely inhibited by activation of 5-HT autoreceptors at this site.

Methiothepin was found to act as a competitive antagonist to RU24969, 5-CT and sumatriptan in the SCN. Detailed concentration-response studies showed that the pK_B for methiothepin appears constant when measured against 5-CT or RU24969 in the SCN with values of 7.9 and 8.1 respectively; this is in excellent agreement with the value of 7.9 in rat cerebral cortex determined by Limberger *et al.* (1991), and a K_d value of 7.3 in ligand binding studies in rat brain (Hoyer & Middlemiss, 1989). Isamoltane, the putative 5-HT $_{1B}$ antagonist (Schoeffer & Hoyer, 1989) was able to antagonize the effects of TFMPP and RU24969 in the SCN, but it was not established whether this antagonism was competitive.

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It is interesting that agonists of the 5-HT $_{1B}$ receptor with distinct potency and differing efficacy in the SCN, appear to be approximately equipotent and equally efficacious in the dorsal raphe nucleus. In the DRN, antagonist studies could only at best be qualitative, because full agonist action could not be obtained. Methiothepin blocked the effect of 5-CT in the DRN, and this is in accord with it having affinity for 5-HT $_{1A}$ receptors (Hoyer & Middlemiss, 1989). Isamoltane (see above), was not an antagonist of the effect of ipsapirone in the DRN, while NAN 190 (Glennon *et al.*, 1988), a putatively selective 5-HT $_{1A}$ antagonist, did antagonize the inhibitory effect of ipsapirone on stimulated 5-HT overflow. Sprouse & Aghajanian (1986) reported that (–)-propranolol could antagonize the inhibition of spontaneous firing in the DRN caused by 5-HT. In this study, (–)-propranolol while having no effect on stimulated 5-HT overflow on its own, could block the effect of ipsapirone in preventing release of endogenous 5-HT. Further characterization of the 5-HT $_{1A}$ receptor may require the development of more selective antagonists of 5-HT receptor subtypes.

Conclusions

We have shown that 5-HT autoreceptors in the SCN can be characterized by measuring agonist potency and antagonist affinity, at an anatomically precise location in slices prepared from rat brain. Our results are in good agreement with quantitative studies with radiolabelled 5-HT when these are made in the absence of reuptake inhibitors. Our results confirm that a wide range of agonists is available for characterization of 5-HT $_{1B}$ receptors on axon terminals, but we conclude that characterization of somatodendritic 5-HT autoreceptors in the rat requires further study.

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