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The role of 5-$HT_{1B/1D}$ receptors in the modulation of 5-hydroxytryptamine levels in the frontal cortex of the conscious guinea pig

Claire Roberts *, Gary W. Price, Brian J. Jones

SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

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Abstract

The role of 5-$HT_{1B/1D}$ receptors in modulating extracellular 5-hydroxytryptamine (5-HT) levels in the guinea pig was investigated with the non-selective 5-$HT_{1B/1D}$ receptor inverse agonist, methiothepin, and the selective 5-$HT_{1B/1D}$ receptor partial agonists, GR 127935 (*n*-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazole-3-yl)[1,1′-biphenyl]-4-carboxamide) and GR 125743 (*n*-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-3-methyl-4-(4-pyridinyl)benzamide). Extracellular 5-HT levels were measured using the technique of brain microdialysis, in the frontal cortex of the freely moving guinea-pig. Extracellular 5-HT was tetrodotoxin sensitive and calcium dependent, and increased when perfused with a high concentration of K^+. In addition, extracellular 5-HT levels were lowered by the 5-$HT_{1B/1D}$ receptor agonist, sumatriptan, and the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin, while perfusion of the selective serotonin re-uptake inhibitor, paroxetine, increased 5-HT in a concentration-dependent manner. Perfusion of methiothepin, GR 127935 and GR 125743 into the frontal cortex caused significant but transient increases of extracellular 5-HT. However, systemic administration of methiothepin, GR 127935 and GR 125743, at 0.3 mg/kg i.p., produced significant decreases in extracellular 5-HT, to minima of $27 \pm 3\%$, $31 \pm 12\%$ and $27 \pm 13\%$ of basal, respectively. The increase of extracellular 5-HT, following 5-$HT_{1B/1D}$ receptor inverse and partial agonist perfusion into the frontal cortex, was probably a consequence of attenuation of an endogenous 5-HT tone at terminal 5-HT autoreceptors. The unexpected decrease in 5-HT levels following systemic administration may be a result of additional attenuation of endogenous 5-HT tone at cell body autoreceptors in the raphe. Such an increase in local 5-HT levels could then stimulate 5-HT_{1A} receptors to inhibit cell firing and hence decrease 5-HT levels in the terminal regions. This was confirmed when co-administration of the 5-HT_{1A} receptor antagonist, WAY 100635, significantly attenuated the GR 127935 decrease in 5-HT.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Microdialysis; 5-$HT_{1B/1D}$ receptor; GR 127935; GR 125743; (Guinea pig)

1. Introduction

5-Hydroxytryptamine (5-HT) release is subject to regulation by a negative feedback system, mediated by autoreceptors located on neuronal terminals and cell bodies. It has been established that the terminal autoreceptor in the rat is of the 5-HT_{1B} receptor subtype (Engel et al., 1986) whereas in other species, including guinea pig and human, the terminal autoreceptor is of the 5-HT_{1D} receptor subtype (Middlemiss et al., 1988; Hoyer and Middlemiss, 1988; Roberts et al., 1994b, 1996). These receptors are now acknowledged to be species homologues of the same gene product and it has recently been suggested that they be known as r 5-HT_{1B}, g 5-HT_{1B} or h 5-HT_{1B} receptors for rat, guinea pig and human, respectively (Hartig et al., 1996). Activation of these autoreceptors has been demonstrated to decrease the amount of 5-HT released into the synapse, regulating the amount of 5-HT released on each neuronal impulse.

Somatodendritic autoreceptors are now known to encompass both 5-HT_{1A} receptor and 5-HT_{1D} (previously known as 5-$HT_{1D\alpha}$) receptor subtypes (Starkey and Skingle, 1994; Davidson and Stamford, 1995). Activation of 5-HT_{1A} autoreceptors on cell bodies and dendrites of 5-HT neurones produces a decrease in spontaneous firing rates of neurones (De Montigny et al., 1984; Mundey et al., 1992), which results in a decrease in 5-HT released in terminal regions (Crespi et al., 1990). In addition, activa-

* Corresponding author. Tel.: (44-1279) 622-038; Fax: (44-1279) 622-230.

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tion of 5-HT_{1A} receptors decreases somatodendritic release of 5-HT within cell body regions (O'Connor and Kruk, 1992; Skingle et al., 1993a; Starkey and Skingle, 1994; Craven et al., 1995; Davidson and Stamford, 1995). Stimulation of 5-HT_{1D} receptors in the raphe nuclei also decreases the amount of 5-HT released in cell body regions (Starkey and Skingle, 1994; Davidson and Stamford, 1995) but does not appear to directly modulate the firing activity of neurones (Craven et al., 1995).

It is unclear how these multiple subtypes of 5-HT autoreceptors interact with one another in vivo. Is the functional activity of one autoreceptor more dominant than another? Blier et al. (1990) previously suggested that there were differences in autoreceptor control between brain regions using the technique of in vivo voltammetry. They demonstrated that the 5-HT release in the frontal cortex was predominantly regulated by somatodendritic 5-HT_{1A} receptors, while the modulation of 5-HT release from the dentate gyrus was predominantly from terminal 5-HT_{1B} receptors.

To help uncover the possible role and importance of 5-$HT_{1B/1D}$ receptors on regulating in vivo 5-HT levels, we have studied the effects of selective and non-selective 5-$HT_{1B/1D}$ receptor ligands on in vivo levels of 5-HT in the frontal cortex. The experiments were carried out using the technique of microdialysis in the freely moving guinea-pig. A preliminary report of this work has previously been published (Roberts et al., 1994a).

2. Materials and methods

2.1. Animals

Male Dunkin Hartley guinea-pigs (*Cavia porcellus*) weighing between 350–450 g were used in all experiments. Animals were maintained on a 12 h light-dark cycle at 22°C, and given free access to food and water.

2.2. Brain microdialysis probes

Dialysis probes were constructed from Perspex blocks ($6.5 \times 4.0 \times 3.00$ mm) with three 7.0 mm long 23 gauge needle tubing (0.64 mm O.D., 0.325 mm I.D.) inserted to create an inlet, outlet and sampling tube. A 3 mm length of polyacrylonitrile dialysis tubing (Hospal, UK, 0.3 mm O.D., 0.2 mm I.D., molecular cut-off 20 kDa) was secured onto the sampling tube and the end sealed with epoxy glue. Fused silica glass capillary tubing (Scientific Glass Engineering, UK) was inserted into the inlet tube, down to the tip of the dialysis membrane. The hole through which the capillary was inserted was sealed with epoxy glue. Probes were made in batches and stored until used. The in vitro recovery of these probes, from a 20 ng/ml 5-HT standard, was $9.6 \pm 0.7\%$ ($n = 7$) when perfused with artificial cerebrospinal fluid, ACSF (NaCl 125 mM; $NaHCO_3$ 27 mM; KCl 2.5 mM; Na_2HPO_4 1.2 mM; NaH_2PO_4 0.5 mM; Na_2SO_4 0.5 mM; $CaCl_2$ 1 mM; $MgCl_2$ 1 mM; glucose 5 mM; pH 7.4) at 2 μl/min at room temperature.

2.3. Brain microdialysis surgery

Guinea pigs were anaesthetised with 2% methoxyflurane delivered with O_2 (1 l/min) and N_2O (3 l/min) in an induction chamber. On attaining surgical anaesthesia the guinea pigs were transferred to a stereotaxic frame (David Kopf) which had been adapted to accommodate an anaesthetic mask and scavenging unit (Klapwyk et al., 1995). Anaesthesia was maintained on 1–2% methoxyflurane with 1 l/min O_2 : 2 l/min N_2O.

Dialysis probes were implanted into the frontal cortex (co-ordinates from the intra-aural zero: AP +15.0 mm, ML ±2.0 mm and 3.0 mm vertical from the dura, using the atlas of Rapisarda and Bacchelli (1977). Probes were secured with two skull screws and dental acrylic cement, and the wound sealed. Animals were allowed 24 h for recovery, after which the probes were perfused with ACSF at a rate of 2 μl/min. Under calcium-free conditions the ACSF was modified by omitting $CaCl_2$ and increasing the NaCl concentration to 127 mM. Under high K^+ conditions ACSF was modified by decreasing the NaCl concentration to 77.5 mM and concurrently increasing the KCl concentration to 50 mM.

After 2 h perfusion, samples were collected every 20 min into 10 μl of 0.4 M perchloric acid. Three samples were taken to measure basal extracellular levels of 5-HT before drug treatment. Following drug treatments, 5-HT levels were measured for at least nine samples.

2.4. High performance liquid chromatography (HPLC) analysis of 5-HT

45 μl dialysis samples were injected onto an HPLC system using centre loop filling, and 5-HT separated from other substances using reverse-phase, ion-pair chromatography. Separation was achieved at a flow rate of 1 ml/min with a 4.6×7.5 mm ODS2, 3 μm column (HPLC Technology) and a mobile phase consisting of 0.15 M NaH_2PO_4, 0.8 mM sodium octanylsulphate, 0.4 mM EDTA, pH 3.8 and 14–16% methanol. The mobile phase was filtered through a 0.22 μm GS filter and degassed with helium.

Detection of 5-HT was performed with an Antec electrochemical detector (ECD), with a glassy carbon working electrode set at +0.65 V versus an Ag/AgCl reference electrode. The detection of 5-HT was linear over the range 2–2000 fmol with a limit of detection, under these conditions, of 2 fmol/sample.

2.5. Drug administration

Guinea pigs were injected intraperitoneally (i.p.) or subcutaneously (s.c.) with vehicle or drug after the third

dialysis sample (i.e. 60 min from the start of the experiment); dialysis samples were then collected for at least 180 min.

When drugs were delivered through the dialysis probe solutions of vehicle or drug were perfused for 120 min following three basal samples, before returning to ACSF for at least 60 min. Drug solutions were switched by hand.

During tetrodotoxin and Ca^{2+}-free conditions, three basal samples were taken before drug solutions were perfused for the duration of the experiment. In the case of the K^+ studies, three basal samples were taken before the modified solution was perfused for a 20 min pulse, returning to control ACSF for the remainder of the experiment. Drug solutions were switched by hand.

2.6. Data analysis

Data from experiments were reported as area under chromatogram peaks. The first three samples were averaged to yield a basal level of extracellular 5-HT. All samples were expressed as a percentage of basal levels. In the majority of experiments percentage of basal values for the individual time points post-treatment were accumulated and averaged, producing a value for the mean percentage of basal, which was an estimate of the mean area under the curve (AUC). Statistical comparisons of mean AUC following drug administration were calculated on a SAS-Research Scientist Application (v. 1.4, release 6.08 (1992), SAS Institute, Cary, NC, USA). For normally distributed data, analyses were performed using a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey-Kramer *t*-test. For non-normally distributed data the non-parametric Mann-Whitney *U*-test was employed. Significance was taken at the 5% level in both cases.

2.7. Materials

GR 127935 (*n*-[4-methoxy-3-(4-methyl-1-piperizinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazole-3-yl)[1,1′-biphenyl]-4-carboxamide), GR 125743 (*n*-[4-methoxy-3-(4-methyl-1-piperizinyl)phenyl]-3-methyl-4-(4-pyridinyl)-benzamide), WAY 100635, paroxetine and sumatriptan were synthesised at SmithKline Beecham Pharmaceuticals. Tetrodotoxin and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) were supplied by Sigma (Poole, UK). Methiothepin mesylate was supplied by Research Biochemicals International (Natick, MA, USA).

3. Results

3.1. Characterisation

Mean basal levels of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), over the 320 min period were 28 ± 6 fmol/sample ($n = 32$) and 474 ± 28 fmol/sample ($n = 32$), respectively. When ACSF perfusing the frontal cortex was switched to incorporate tetrodotoxin (1 μM) or Ca^{2+}-free ACSF, 5-HT levels were significantly reduced to less than 10% of basal ($P < 0.05$). When ACSF was changed for a 20 min pulse of ACSF containing 50 mM K^+, 5-HT levels were significantly increased to a maximum of 253 ± 39% of basal before returning to pre-stimulated levels ($P < 0.05$). When data are summarised as mean AUC, Ca^{2+}-free, tetrodotoxin and high K^+ gave 54 ± 8, 55 ± 4 and 131 ± 3% ($n = 3$), respectively.

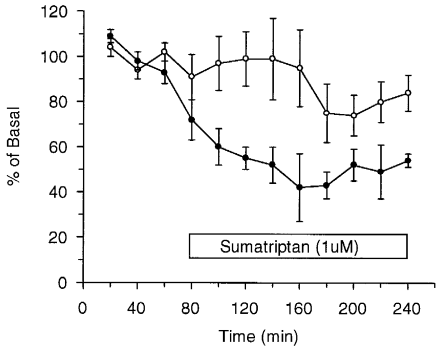

Fig. 1. Effect of (○) vehicle ($n = 8$) and (●) 1 μM sumatriptan ($n = 6$) when perfused down the dialysis probe into the frontal cortex. When the data are summarised as mean AUC post-treatment, sumatriptan significantly reduces the levels of 5-HT ($P < 0.05$, ANOVA, Tukey-Kramer *t*-test).

The $5\text{-HT}_{1B/1D}$ receptor agonist, sumatriptan (1 μM), when perfused down the dialysis probe into the frontal cortex produced a significant decrease in 5-HT levels ($P < 0.05$), reaching a minimum of 42 ± 15% of basal ($n = 5$) after a 2 h period (Fig. 1). The mean AUC was 52 ± 8% ($n = 6$).

The 5-HT_{1A} receptor agonist, 8-OH-DPAT, when injected at a dose of 1 mg/kg s.c. (Gurling et al., 1994), produced a gradual and significant decrease in extracellular 5-HT ($P < 0.05$), reaching a minimum of 34 ± 16% of

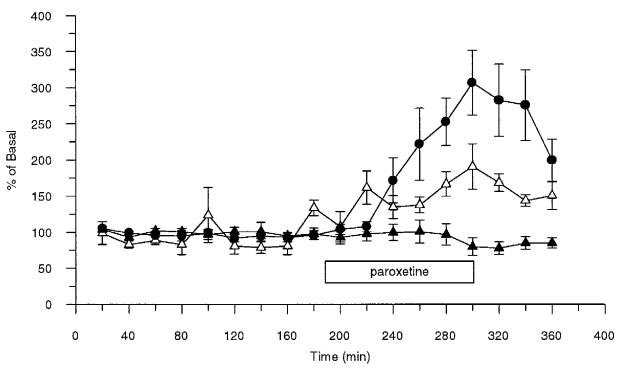

Fig. 2. The effect of (▲) vehicle ($n = 8$), (△) 5 μM paroxetine ($n = 4$) and (●) 10 μM paroxetine ($n = 4$) when perfused into the frontal cortex. When the data are summarised as mean percent basal, both 5 μM and 10 μM paroxetine treatment were significantly greater than vehicle ($P < 0.05$, ANOVA, Tukey-Kramer *t*-test).

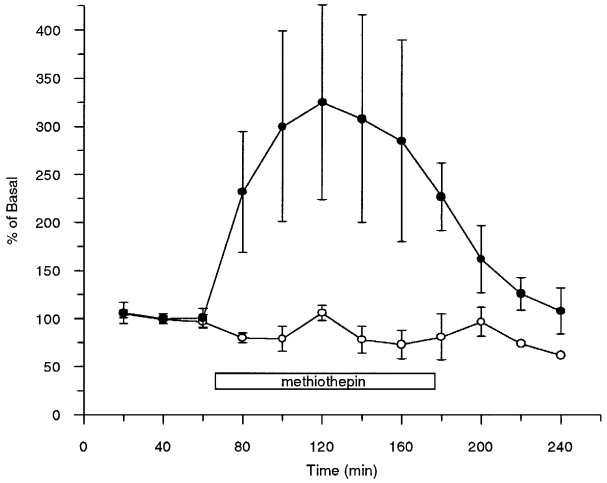

Fig. 3. Effect of (○) vehicle ($n = 4$) and (●) 10 μM methiothepin ($n = 7$) on 5-HT levels in the frontal cortex of the freely moving guinea-pig. Drugs were perfused through the dialysis probe, directly into the frontal cortex. When the data are summarised as mean AUC, methiothepin treatment is significantly greater than vehicle (* $P < 0.05$, Mann-Whitney *U*-test).

basal ($n = 4$) 3 h after dosing. The mean AUC was $66 \pm 3\%$ ($n = 4$).

Paroxetine, when administered down the dialysis probe into the frontal cortex, increased 5-HT levels in a concentration-dependent manner (Fig. 2). 5 μM paroxetine increased 5-HT levels to a maximum of $191 \pm 31\%$ of basal ($n = 4$) 2 h after treatment with a mean percentage of basal of $153 \pm 9\%$ ($n = 4$). 10 μM paroxetine increased 5-HT levels even further, reaching $307 \pm 45\%$ of basal ($n = 9$) at the 2 h post-treatment period with a mean percentage of basal of $208 \pm 18\%$ ($n = 9$).

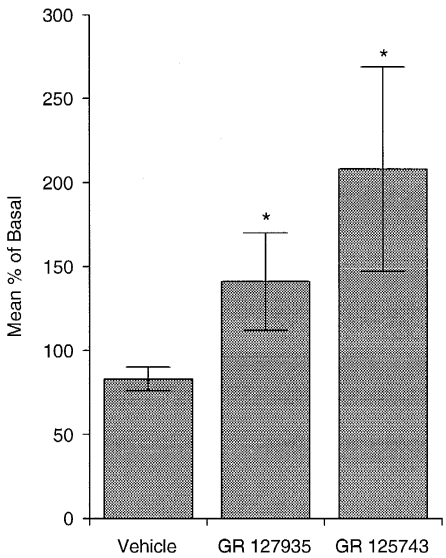

Fig. 4. Effect of perfusing vehicle ($n = 4$), 10 μM GR 127935 ($n = 3$) and 10 μM GR 125743 ($n = 4$) on 5-HT levels in the frontal cortex of the freely moving guinea-pig. Data are summarised as mean AUC post-treatment. Both treatments are significantly different from vehicle ($^{*}P < 0.05$, Mann-Whitney *U*-test).

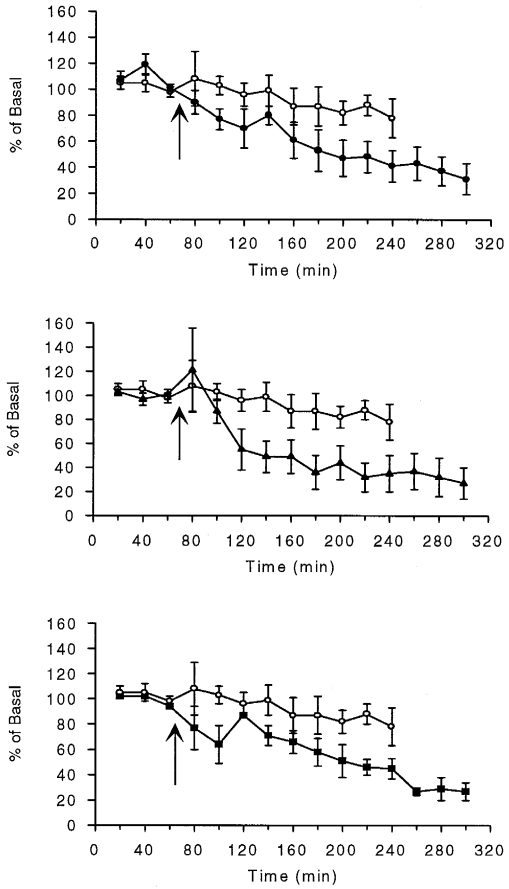

Fig. 5. Effect of (○) vehicle ($n = 12$), (●) 0.3 mg/kg i.p. methiothepin ($n = 2$), (▲) 0.3 mg/kg i.p. GR 127935 ($n = 6$) or (■) 0.3 mg/kg i.p. GR 125743 ($n = 6$) on 5-HT levels in the frontal cortex of the freely moving guinea-pig. When $n = 2$, data are reported as mean ± standard deviation. When data are summarised as mean AUC, both GR 127935 and GR 125743 were significantly different from vehicle (* $P < 0.05$, ANOVA, Tukey-Kramer *t*-test).

3.2. Effect of 5-HT$_{1B/1D}$ receptor partial and inverse agonists on 5-HT levels

When the non-selective 5-HT$_{1B/1D}$ receptor inverse agonist, methiothepin (10 μM), was perfused down the dialysis probe directly into the frontal cortex, a significant increase in 5-HT levels was observed, reaching a maximum of $325 \pm 101\%$ of basal ($n = 7$) 1 h after application (Fig. 3). The mean AUC was $293 \pm 92\%$ ($n = 7$).

The selective 5-HT$_{1B/1D}$ receptor partial agonists GR 127935 (10 μM) and GR 125743 (10 μM) also produced significant transient increases in extracellular 5-HT, reaching maxima of $189 \pm 50\%$ ($n = 4$) and $270 \pm 57\%$ ($n = 3$) of basal, respectively. The mean AUCs were $141 \pm 29\%$ ($n = 4$) and $208 \pm 61\%$ ($n = 3$) for GR 127935 and GR 125743, respectively (Fig. 4).

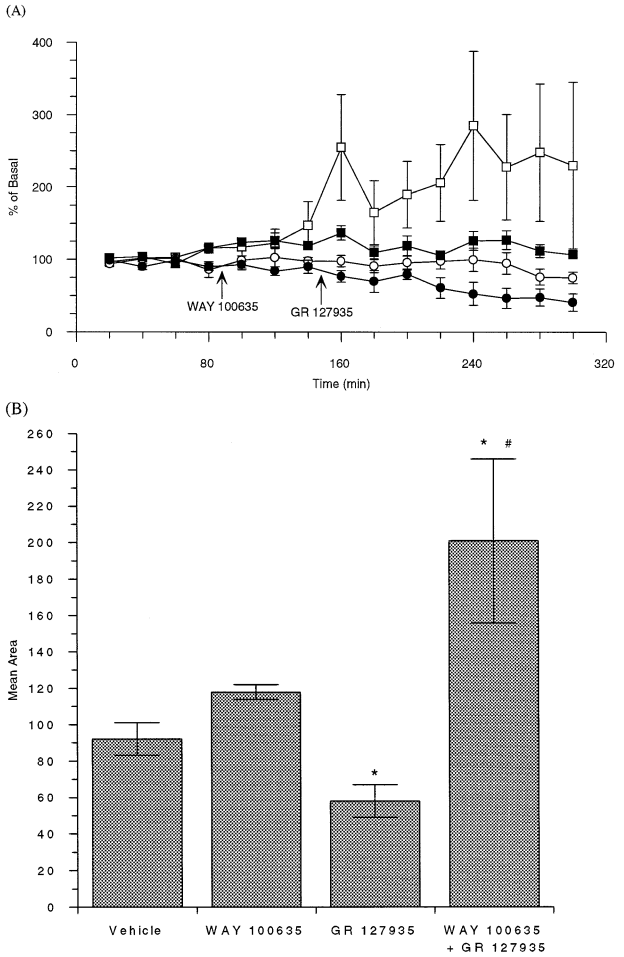

Fig. 6. (A) Effect of systemic administration of (○) vehicle ($n = 9$), (■) 1 mg/kg i.p. WAY 100635 ($n = 3$), (●) 0.3 mg/kg i.p. GR 127935 ($n = 6$) and (□) 0.3 mg/kg i.p. GR 127936 in the presence of 1 mg/kg i.p. WAY 100635 ($n = 7$). (B) Data are summarised as mean percentage of basal ± standard deviation. Both GR 127935 and GR 127935 in the presence of WAY 100635 were significantly different from vehicle ($^{\#}$ $P < 0.05$, ANOVA, Tukey-Kramer). In addition, GR 127935 in the presence of WAY 100635 was significantly different from GR 127935 treatment alone (* $P < 0.05$, ANOVA, Tukey-Kramer).

However, when administered systemically, methiothepin (0.3 mg/kg i.p., $n = 2$), GR 127935 (0.3 mg/kg i.p., $n = 6$) and GR 125743 (0.3 mg/kg i.p., $n = 6$) decreased extracellular 5-HT, the latter two drug effects being significant (Fig. 5), reaching minima of 27 ± 3% ($n = 2$), 31 ± 12% ($n = 6$) and 27 ± 13% ($n = 6$) of basal with mean AUCs of 54 ± 6% ($n = 2$), 58 ± 9% ($n = 6$) and 52 ± 8% ($n = 6$), respectively.

Pre-treatment with WAY 100635 (1 mg/kg i.p.) significantly ($P < 0.05$) attenuated the GR 127935 (0.3 mg/kg i.p.) inhibition of extracellular 5-HT, reaching a maximum of 285 ± 103% of basal. WAY 100635 had no significant effect on 5-HT levels alone (Fig. 6).

4. Discussion

4.1. Characterisation

The observation that basal extracellular levels of 5-HT, in the frontal cortex of the freely moving guinea-pig, are sensitive to both tetrodotoxin and Ca^{2+} depletion indicates the neuronal origin and physiological release of 5-HT. This was confirmed by the fact that basal 5-HT can also be elevated by a high K^{+} concentration and a range of paroxetine concentrations, and inhibited by the 5-HT_{1A} and 5-$HT_{1B/1D}$ receptor agonists 8-OH-DPAT and sumatriptan, consistent with somatodendritic and terminal autoreceptor activation, respectively. These findings have been reported in the guinea pig by several independent groups (Sleight et al., 1990; Lawrence and Marsden, 1992; Skingle et al., 1993b, 1995). Two important findings have arisen from this work: (a) about 70% of the basal 5-HT levels were susceptible to both 5-HT_{1A} and 5-$HT_{1B/1D}$ receptor agonist modulation, and (b) extracellular 5-HT could be significantly elevated with the selective serotonin re-uptake inhibitor, paroxetine.

4.2. Effect of 5-$HT_{1B/1D}$ receptor partial and inverse agonists

Perfusion of 5-$HT_{1B/1D}$ receptor partial and inverse agonists directly into the frontal cortex produced significant increases in 5-HT levels. This increase in 5-HT is probably due to attenuation of endogenous 5-HT tone at the 5-HT terminal autoreceptor. This is consistent with in vitro [^{3}H]5-HT studies, where several groups have concluded that the 5-HT terminal autoreceptor in the guinea pig is of the 5-$HT_{1B/1D}$ receptor subtype (Middlemiss et al., 1988; Limberger et al., 1992; Roberts et al., 1994b, 1996). The finding with methiothepin has been confirmed by several groups (Hogg and Hutson, 1994; Hutson et al., 1995; Moret and Briley, 1995a,b) but has been refuted by another (Gardier et al., 1992). Similarly there are conflicting results with GR 127935 (Hogg and Hutson, 1994; Hutson et al., 1995; Moret and Briley, 1995a,b; Skingle et al., 1995). However, a group that reports an increase with methiothepin does not necessarily see a comparable increase with GR 127935. Methiothepin is known to have affinity for many receptors, e.g. α_1-adrenoceptors, which may account for effects that differ from the 5-$HT_{1B/1D}$ receptor partial agonists. In addition, discrepancies between groups could be introduced with the use of different tissue regions and freely moving versus anaesthetised animals.

From the data reported here it is apparent that there was a large variation in the increase of 5-HT levels seen after perfusion of methiothepin down the dialysis probe and that it was transient in nature. This drug response was surpris-

ing but could imply that there are (i) non-specific actions of the drug or (ii) some compensatory mechanisms in action, limiting the increase of 5-HT at the synapse. A possible explanation for the large variations in response is that the degree of tone at the 5-HT terminal autoreceptor in vivo is variable. The reason for this is unclear but it may depend on the level of activity and behaviour of the animal during the experiment.

When the 5-HT_{1D} receptor antagonists were administered systemically, instead of the expected increase in 5-HT levels significant decreases in neurotransmitter levels were observed. The drug doses used were extrapolated from data obtained from behavioural studies, at which full blockade of a 5-HT_{1D} receptor agonist effect was achieved (induced hypothermia, Hatcher et al., 1995). At these doses the degree of inhibition of 5-HT levels were comparable for all three drugs. This phenomenon has also been recently reported by another independent group (Skingle et al., 1994).

The reason for this paradoxical decrease in 5-HT levels is unknown. It may be due to a specific property of the compounds tested. These compounds possess the unwanted characteristics of α_1 and 5-HT_2 activity (Scopes et al., 1994) which could elicit a decrease in 5-HT levels. In addition, both GR 127935 (Watson et al., 1995) and GR 125743 (unpublished observation) possess some degree of partial agonism in a recombinant cell line expressing functional human 5-HT_{1B} receptors. However, methiothepin fails to demonstrate intrinsic activity even in this high receptor expression system (Watson et al., 1995). In addition, GR 127935 fails to demonstrate intrinsic activity at terminal autoreceptors (Roberts et al., 1994b, 1996) and is unlikely to affect firing rate, since it has been demonstrated that 5-$HT_{1B/1D}$ receptor activation in the raphe has no effect on cell firing rate (Craven et al., 1995). Therefore, it is unlikely that the partial agonist activity of GR 127935 will lead to the above effects on 5-HT release.

Alternatively the decrease observed may be a consequence of antagonism at 5-$HT_{1B/1D}$ receptors elsewhere in the central nervous system, in addition to terminal autoreceptor blockade. A plausible explanation is that antagonism of inhibitory 5-HT_{1D} receptors on raphe cell bodies (see Section 1 for evidence of raphe 5-HT_{1D} receptors) leads to an increase of extracellular 5-HT at the site that stimulates somatodendritic 5-HT_{1A} receptors, resulting in a decrease in cell firing and hence an overall decrease of 5-HT release from terminal regions. The attenuation of the GR 127935 inhibition of 5-HT with WAY 100635 confirms this hypothesis. However, attenuation of 5-$HT_{1A/1B/1D}$ receptors also resulted in an increase in extracellular 5-HT, significantly higher than vehicle levels. This observed increase in 5-HT levels was highly variable, e.g. a maximum effect of 285 $\pm$ 103% of basal. However, one would anticipate that if all mechanisms to control extracellular 5-HT were removed, then the resultant 5-HT level would vary dramatically between animals, depending on factors like animal activity and behaviour. It was surprising that the mixed 5-$HT_{1A/1B/1D}$ receptor inverse agonist, methiothepin, did not produce a similar effect as co-administration of the 5-HT_{1A} receptor antagonist, WAY 100635, and a 5-$HT_{1B/1D}$ receptor partial agonist, GR 127935. This discrepancy may be due to the additional receptor activities of methiothepin, e.g. adrenergic and histamine activity.

4.3. Clinical implications

Brain 5-HT levels are thought to be important in the pathogenesis of depression (see following references for evidence: Fries, 1954; Ayd, 1957; Delay et al., 1952; Kuhn, 1958; Shopsin et al., 1975). This was confirmed by Delgado et al. (1991) who demonstrated that acute lowering of plasma tryptophan (the precursor of 5-HT) also resulted in a reversal of the therapeutic effect of several types of antidepressant drugs.

Current therapies for this disorder, for example selective 5-HT reuptake blockers, have been suggested by Blier et al. (1987) to exert their therapeutic action by increasing synaptic 5-HT levels, which then desensitise 5-HT terminal autoreceptors. Clinical efficacy of these drugs takes between 2–3 weeks, the time reported to take for desensitisation of 5-HT autoreceptors. Therefore, one would predict that blockade of 5-HT terminal autoreceptors would produce a rapidly acting antidepressant.

If the decrease in 5-HT levels reported in this study is a generic effect of 5-$HT_{1B/1D}$ receptor antagonism, then the strategy for increasing 5-HT levels at terminal regions will depend on separating the pharmacology of raphe cell body 5-HT_{1D} receptors from terminal 5-HT_{1B} receptors. If this is not possible then the approach will have to rely on either (i) the 5-HT_{1A} receptor desensitising, on which there are many conflicting publications in the literature (Kreiss and Lucki, 1992; Sharp et al., 1993; Hjorth and Auerbach, 1994; Invernizzi et al., 1994) or (ii) the use of combinations of 5-$HT_{1B/1D}$ and 5-HT_{1A} receptor antagonists. A similar strategy has already been adopted by Artigas et al. (1994), combining 5-HT reuptake inhibitors and 5-HT_{1A} receptor antagonist administration in the treatment of depression. Preliminary reports have indicated that co-administration of a 5-HT_{1A} receptor antagonist (pindolol) decreased the delay taken for a clinically effective antidepressant effect with 5-HT reuptake inhibitors. Extrapolating from the data reported in this paper, for co-administration of a 5-HT_{1A} and 5-$HT_{1B/1D}$ receptor antagonist, one would predict a similar antidepressant action, with a quick onset of action.

4.4. Summary

Therefore to conclude, local perfusion of 5-$HT_{1B/1D}$ receptor inverse and partial agonists attenuates 5-HT terminal autoreceptor function in vivo. However, when these

compounds were administered systemically, this effect was overridden, probably as a consequence of modulation of cell firing resulting from concurrent 5-HT_{1D} receptor antagonism at 5-HT neuronal cell bodies. Co-administration of a 5-$HT_{1B/1D}$ receptor partial agonist in the presence of a 5-HT_{1A} receptor antagonist removed this limiting effect to reveal an increase in extracellular 5-HT levels.

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