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Diurnal variation in 5-HT_{1B} autoreceptor function in the anterior hypothalamus *in vivo*: effect of chronic antidepressant drug treatment

[1]Tamsin J.O. Sayer, [1]Serina D. Hannon, *[,2]Peter H. Redfern & [1]Keith F. Martin

[1]Knoll Pharmaceuticals Research Department, Nottingham, NG2 3AA and [2]Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY

1 Intracerebral microdialysis was used to examine the function of the terminal 5-hydroxytryptamine (5-HT) autoreceptor in the anterior hypothalamus of anaesthetized rats at two points in the light phase of the light–dark cycle.

2 Infusion of the 5-$HT_{1A/1B}$ agonist 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridyl)-1H-indole (RU24969) 0.1, 1.0 and 10 μM through the microdialysis probe led to a concentration-dependent decrease (49, 56 and 65% respectively) in 5-HT output. The effect of RU24969 (1 and 5 μM) was prevented by concurrent infusion of methiothepin (1 and 10 μM) into the anterior hypothalamus *via* the microdialysis probe. Infusion of methiothepin alone (1.0 and 10 μM) increased (15 and 142% respectively) 5-HT output.

3 Infusion of RU24969 (5 μM) through the probe at mid-light and end-light resulted in a quantitatively greater decrease in 5-HT output at end-light compared with mid-light.

4 Following treatment with either paroxetine hydrochloride (10 mg kg^{-1} i.p.) or desipramine hydrochloride (10 mg kg^{-1} i.p.) for 21 days the function of the terminal 5-HT_{1B} autoreceptor was more markedly attenuated at end-light.

5 The data show that, as defined by the response to RU24969, the function of the 5-HT_{1B} receptors that control 5-HT output in the anterior hypothalamus is attenuated following chronic desipramine or paroxetine treatment in a time-of-day-dependent manner.

Keywords: 5-HT release; 5-HT_{1B} receptors; hypothalamus; intracerebral microdialysis; paroxetine; desipramine

Abbreviations: aCSF, artificial cerebrospinal fluid; HPLC-ECD, high performance liquid chromatography with electrochemical detection; 5-HT, 5-hydroxytryptamine; MAOI, monoamine oxidase inhibitor; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; RU24969, 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridyl)-1H-indole; SSRI, selective serotonin reuptake inhibitor

Introduction

In the brain, 5-hydroxytryptamine (5-HT) is a promiscuous neurotransmitter, exerting its effects through a wide range of distinct receptors divided into 5-HT_1 to 5-HT_7 subtypes (Hoyer *et al*., 1994). The 5-HT_1 division is perhaps the most well documented and is further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E} and 5-HT_{1F} receptors (Hoyer *et al*., 1994). Neuronal release of 5-HT is under the control of two types of presynaptically located autoreceptors (Cerrito & Raiteri, 1979; Verge *et al*., 1985), which have been classified as 5-HT_{1A} receptors on the cell body and 5-HT_{1B} receptors on the nerve terminal in the rat brain (Martin & Sanders-Bush, 1982; Middlemiss, 1984; 1985; Verge *et al*., 1985; Martin & Marsden, 1986). There is some evidence that 5-$HT_{1B/1D}$ receptors are also found on 5-HT cell bodies, where they may be involved in controlling dendritic release of 5-HT (Starkey & Skingle, 1994; Davidson & Stamford, 1995).

For over 40 years 5-HT has been primarily implicated in the aetiology of depression, and there is clinical evidence to support this contention. For example, there is a significant decrease in plasma tryptophan levels in depressives. Cowen *et al*. (1989); Pietraszek *et al*. (1991) and Delgado *et al*. (1990) have shown that rapid tryptophan depletion precipitated a relapse in those depressed inpatients who were in remission. The number of 5-HT transporter sites is decreased in the cortex and hippocampus of depressed suicide victims (Leake *et al*., 1991; Little *et al*., 1993) and the density of 5-HT_2 and 5-HT_{1D} receptors in the frontal cortex has been reported to be increased in depressed suicide victims (Stanley & Mann, 1983; Lowther *et al*., 1991).

Since the discovery of the terminal 5-HT autoreceptor, and given the fact that many clinically effective antidepressant drugs target the 5-HT system, several authors have investigated the effect of chronic antidepressant drug treatment on terminal 5-HT autoreceptor function. However, both *in vivo* and *in vitro* results of these studies have been conflicting. Sleight *et al*. (1989), using *in vivo* microdialysis, found no evidence for autoreceptor down-regulation in the hippocampus after prolonged administration of either amitriptyline or MDL72394 (a putative monoamine oxidase inhibitor, MAOI). Similarly, Blier *et al*. (1988), using an indirect measure of receptor function and a non-specific antagonist, reported that terminal 5-HT autoreceptor function in the hippocampus was unaffected by chronic clorgyline treatment. By contrast, Blier *et al*. (1988) and Chaput *et al*. (1986), using the same methodology, strain of animal, experimental protocol and brain region, have shown that the 5-HT autoreceptor is down-regulated after either repeated fluoxetine or citalopram treatment. On the other hand, *in vitro* studies have

*Author for correspondence.

demonstrated autoreceptor down-regulation in the hippocampus (Blier & Bouchard, 1994), hypothalamus (Moret & Briley, 1990; Blier & Bouchard, 1994) but not the frontal cortex (Blier & Bouchard, 1994) of rats and guinea-pigs following treatment with the selective serotonin reuptake inhibitors (SSRI) citalopram or paroxetine. Conversely, befloxamine (a reversible inhibitor of monoamine oxidase, RIMA) administration had no effect on 5-HT autoreceptor function in the hypothalamus, hippocampus or frontal cortex of the guinea-pig (Blier & Bouchard, 1994). The results of these studies illustrate that different classes of antidepressant drugs do not appear to have a common effect on terminal 5-HT autoreceptor function.

One important factor which all the studies cited above have overlooked is the marked circadian rhythm in the 5-HT system (for a comprehensive review see Martin & Redfern, 1997). Therefore, we have determined whether the terminal 5-HT$_{1B}$ autoreceptor function varies with the time of day and whether this influences the effects of either chronic paroxetine or desipramine treatment in the rat hypothalamus.

Methods

Animals

Male Wistar rats (Olac, Bicester) weighing 240–260 g were used throughout the study. The animals were housed in pairs under a 12:12 light-dark cycle (lights on 06.00 h), at an ambient temperature of 21°C and had free access to food and tap water.

Implantation of dialysis probes

Rats were anaesthetized with chloral hydrate (600 mg kg^{-1} i.p.) and supplementary doses of anaesthetic (30 mg i.p.) were given as needed during the course of the experiment. The animals were placed in a stereotaxic frame and concentric microdialysis probes (CMA/12 microdialysis probe, 2 mm long membrane and 0.5 mm o.d., Carnegie Medicin, Biotech Instruments) implanted into the anterior hypothalamus (coordinates with reference to Bregma and the skull surface AP −1.3 mm, ML −0.6 mm and depth −9.3 mm, according to the atlas of Paxinos & Watson, 1982). The probe was continuously perfused, at a rate of 1 μl min^{-1} (model 22 Microinjection pump, Harvard Apparatus or CMA/100 Microinjection pump, Carnegie Medicin, Biotech Instruments) with artificial cerebrospinal fluid (aCSF) (composition in mM: NaCl 147; KCl 4; $CaCl_2$ 4 pH 7.4) containing the selective 5-HT reuptake inhibitor citalopram (1 μM), and the effluent collected onto ice. At the end of the experiment the brain was removed and the position of the probe was visually confirmed. Before implantation the recovery of 5-HT from the probe in the dialysis solution was checked *in vitro* at the flow rate used; probes with recoveries of below 15% were discarded.

Experimental protocol

Following a 90 min stabilization period dialysate samples were collected every 15 min. Two aliquots were taken to serve as pre-intervention controls and successive 15 min fractions collected throughout the experiment. For agonist studies the drug was infused *via* the probe for 15 min, immediately after the control samples, and six subsequent samples were collected. For antagonist studies, the antagonist alone was infused *via* the probe for 15 min, after the control samples, then the agonist and the antagonist were infused together *via* the probe for a further 15 min. Dialysate samples were assayed immediately for their 5-HT content using reverse phase high performance liquid chromatography coupled to an electrochemical detector (HPLC-ECD). The times at which experiments were carried out are indicated in the appropriate part of the Results section.

Perfusate analysis

The HPLC-ECD system used has been described previously (Martin *et al*., 1992). Briefly it consisted of a Hypersil ODS2 column (10 cm × 2 mm i.d.) with 3 μm packing (HPLC Technology) linked to a Coulochem electrochemical detector with dual electrodes, electrode 1 set at +0.1 V and electrode 2 at +0.28 V (model 5011 analytical cell, ESA Inc.). Mobile phase (composition: NaH_2PO_4 0.1 M; sodium octane sulphonic acid 0.93 mM; 0.07% v v^{-1} dibutylamine and 12% v v^{-1} methanol) adjusted to pH 3.0 with orthophosphoric acid, was supplied to the system by a Severn Analytical solvent delivery system (SA6410B Severn Analytical) at a flow rate of 0.45 ml min^{-1}. The chromatogram was plotted on a Shimadzu C-R6A chromatopac integrator (Dyson Instruments Ltd).

Statistical analysis

Values are expressed as a percentage of the control value i.e. average concentration of 5-HT in the two dialysate samples taken immediately prior to any drug infusion. Data was analysed by two-way analysis of variance (ANOVA) to detect differences between treatments, followed by Studentized range test to determine where differences lay between treatments; $P \leqslant 0.05$ was considered significant.

Chronic antidepressant treatment

Rats were treated for 21 days with a once daily injection of either desipramine hydrochloride (10 mg kg^{-1} i.p.) or paroxetine hydrochloride (10 mg kg^{-1} i.p.) or an equivalent volume of saline. The precise time of injection was varied each day to avoid the animals taking the injection as a time cue, but injection was always performed in the afternoon during the rat's light phase. All experiments were performed after a 24 h washout period.

Drugs

All drugs which were used acutely were infused *via* the probe and were made up in aCSF including 1 μM citalopram on the day of use. Chloral hydrate was made up in 0.9% sterile saline every 2 days as required. Pilot studies have indicated that drug transferred out of the dialysis probe with similar efficiencies to the rate of entry (i.e. approximately 15%).

Drugs were purchased from suppliers as follows: NaCl, KCl, orthophosphoric acid and NaH_2PO_4 all Aristar quality (BDH Chemicals), octane sulphonic acid (Kodak Clinical Drugs Ltd.), HPLC grade methanol (Rathburn Chemicals), dibutylamine (Aldrich), chloral hydrate, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) and desipramine (Sigma Chemical Co.).

The following drugs were donated by the companies indicated; RU24969 (Roussel-Uclaf), citalopram (Lundbeck), methiothepin (Hoffmann-LaRoche) and paroxetine (Smith-Kline Beecham).

Results

All experiments were performed during the early to middle stages of the light period (i.e. 0800 to 1300 h) unless otherwise stated.

Basal efflux of 5-HT

The output of 5-HT into the dialysate was stable over the collection period. The mean 5-HT level in the first 30 min of collection was 25.0 ± 0.2 fmole 15 μl^{-1} sample ($n = 12$).

When calcium ions were removed from the perfusing aCSF (composition in mM: NaCl 147, KCl 4, pH 7.4) for 60 min there was a rapid decrease in 5-HT output within the first 15 min of this intervention, reaching a maximum decrease of 98% (2% of control ± 0.6%, $P < 0.01$, $n = 4$) at $t = 45$ min. When calcium ions were re-introduced into the aCSF, 5-HT levels returned rapidly to baseline and increased to a maximum of 121 ± 6.6% of preintervention control at $t = 150$ min (Figure 1).

Modified aCSF containing 100 mM K^+ was infused *via* the probe for 15 min. This led to an immediate and significant increase in 5-HT output of 456% of control ± 11.5% ($P < 0.01$, $n = 7$) in the first fraction and 345% of control ± 8.0% ($P < 0.01$, $n = 6$) in the second fraction; dialysate 5-HT content subsequently returned to baseline (data not shown).

Pharmacological characterization of the hypothalamic 5-HT autoreceptor

8-OH-DPAT is a selective agonist at the 5-HT$_{1A}$ receptor (Middlemiss & Fozard, 1983). It was used because subsequent experiments used RU24969 which is not completely selective for the 5-HT$_{1B}$ receptor, having an almost equal affinity for the 5-HT$_{1A}$ receptor (van Wijngaarden *et al*., 1990). 8-OH-DPAT was infused into the anterior hypothalamus to ensure that any effect of RU24969 produced was due to its activity as a 5-HT$_{1B}$ agonist. A 15 min infusion of 1 μM 8-OH-DPAT *via* the dialysis probe had no significant effect on 5-HT release compared to control animals, see Figure 2a.

Increasing concentrations of RU24969 were infused for 15 min *via* the probe leading to a dose-dependent decrease in 5-HT output. At a concentration of 10 μM, there was a maximum inhibition of 65% (35 ± 2% of control at $t = 30$ min, $P < 0.01$, $n = 6$), 1 μM 56% (44 ± 1.7% of control at $t = 45$ min, $P < 0.01$, $n = 4$) and 0.1 μM had a maximum inhibition of 49%

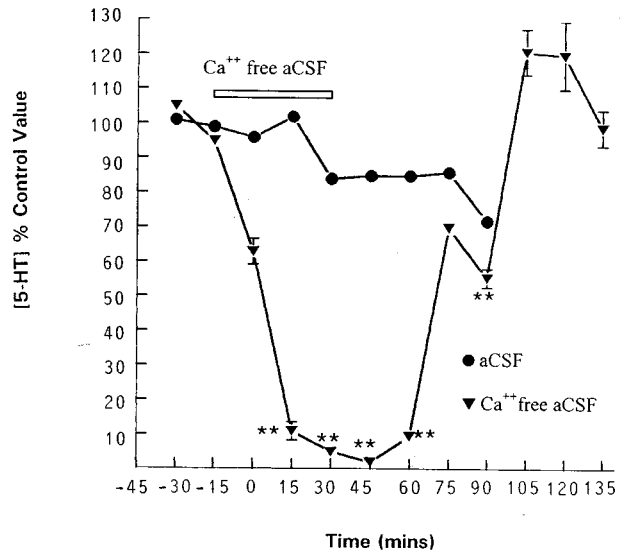

Figure 1 Effect of removing calcium ions from the perfusing medium on the level of 5-HT in dialysate collected from the anterior hypothalamus. Concentric microdialysis probes were perfused with aCSF containing (in mM): NaCl 147, KCl 4, $CaCl_2$ 4 and citalopram 1 μM and samples collected every 15 min. $^{**}P < 0.01$ vs pooled control (two way ANOVA). Data are expressed as a percentage of the concentration of 5-HT in the two dialysate samples taken immediately prior to any intervention (control value). Each point represents the mean value with s.e.mean shown by vertical bars. Control $n = 12$, calcium free buffer $n = 4$.

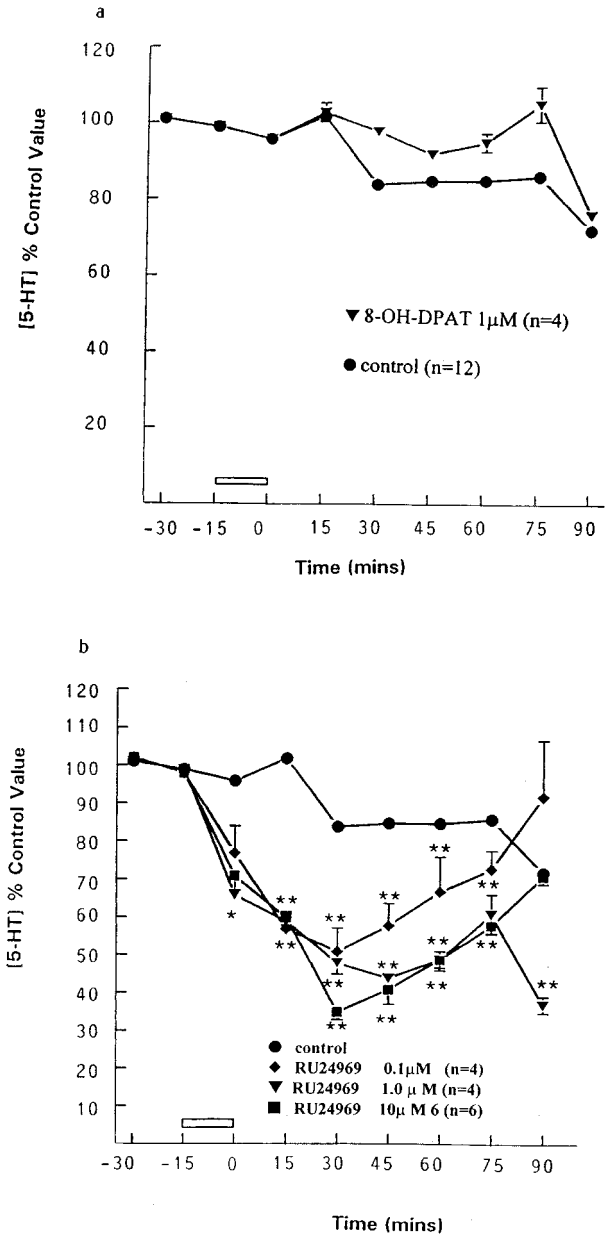

Figure 2 Effects of infusion of (a) the 5-HT$_{1A}$ agonist 8-OH-DPAT and (b) the 5-HT$_{1A/1B}$ agonist RU24969 on 5-HT output in the anterior hypothalamus. (a) 8-OH-DPAT, 1 μM ($n = 4$) was infused for 15 min through the probe; pooled control, $n = 12$. (b) RU24969, 0.1 μM $n = 4$, 1 μM $n = 4$, 10 μM $n = 6$) was infused for 15 min; pooled control, $n = 12$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs pooled control value (two-way ANOVA). Data are expressed as a percentage of the concentration of 5-HT in the two dialysate samples taken immediately prior to any intervention (control value), each point represents mean with the s.e.mean indicated by vertical bars.

(51±6.2% of control at $t=45$ min, $P<0.05$, $n=4$), see Figure 2b.

Methiothepin (metitepin) has been shown to be a 5-HT$_1$ receptor antagonist (Hilbert & Middlemiss, 1986); since no selective 5-HT$_{1B}$ receptor antagonist was available to us, methiothepin (1 μM), was infused for 15 min alone and for a further 15 min with 1 μM RU24969. Methiothepin reversed the RU24969-induced decrease in 5-HT output. Thus in the presence of 1 μM methiothepin, RU24969 (1 μM) caused a maximum inhibition of 5-HT output of 27% (73% of control ±7% of control at $t=30$ min, $n=4$), which was not significantly different from control animals (see Figure 3). Methiothepin (10 μM) also antagonized the effect of 5 μM RU24969, so that the maximum inhibition of 5-HT output was 17% (83 of control ±3.8% of control at $t=30$ min, $n=4$), which was not significantly different from controls, (see Figure 3).

Infusion of methiothepin (1 or 10 μM) alone for 30 min significantly increased 5-HT output, with maximum increases to 115% of preintervention levels (±5.8%) at $t=45$ min ($P<0.05$, $n=4$) and to 242% of preintervention controls (±7.1%) at $t=60$ min ($P<0.01$, $n=5$), (Figure 3) respectively.

Diurnal variation in 5-HT autoreceptor function

Basal 5-HT levels at mid-light and end-light were 38.7±0.3 fmole 15 μl^{-1} sample ($n=4$) and 28.4±0.1 fmole 15 μl^{-1} sample ($n=6$) respectively. There was no significant change in 5-HT release after lights off. The level of 5-HT in the dialysate was significantly higher at mid-light than end-light ($P<0.05$ unpaired Student's t-test).

Release of 5-HT from control animals was stable over the course of the experiment. When 5 μM RU24969 was infused at mid-light, i.e. 12.00 h, it produced a maximal decrease in 5-HT release of 65% (35% of control ±3.3% at $t=45$ min, $P<0.01$, $n=4$), with levels returning to basal by $t=90$ min (Figure 4).

By contrast, infusion of RU24969 at 18.00 h, the time lights went off in the animal colony, significantly attenuated 5-HT release to below the level of detection (1–2 fmole 15 μl^{-1}), $P<0.01$ $n=5$. The effect was maximal 1 h post infusion and 5-HT levels did not return to basal values over the course of the experiment (Figure 4).

Antidepressant effects on 5-HT autoreceptor function at mid-light and end-light

Chronic antidepressant treatment did not affect basal levels of 5-HT (saline-treated 39±8.2 fmole 15 μl^{-1} sample, paroxetine 30.7±4.1 fmole 15 μl^{-1} sample, desipramine 34.4±2.2 fmole 15 μl^{-1} sample) measured at mid-light. When measured at end-light neither prolonged paroxetine nor desipramine treatment affected basal 5-HT levels compared to saline-treated animals (saline-treated 35±9 fmole 15 μl^{-1} sample, paroxetine 34.2±4.5 fmole 15 μl^{-1} sample, desipramine 41.3±5.6 fmole 15 μl^{-1} sample; one-way ANOVA with *post hoc* Studentized range test, $P>0.05$, $n=4-5$).

At mid-light, RU24969 (5 μM) infused into the hypothalamus of rats treated chronically with saline, produced a maximal inhibition of 5-HT release of 51% (49% of control ±1.7% at $t=60$ min, $n=4$). Infusion of RU24969 *via* the dialysis probe in desipramine-treated animals led to a maximal inhibition of 47% (53% of control ±1.7% at $t=45$ min, $n=5$). Paroxetine-treated animals gave a similar result, with a maximal inhibition of 46% (54% of control ±2.0% at $t=60$ min, $n=4$), (Figure 5). However in rats treated with either of the two antidepressant drugs (paroxetine or desipramine), the effects of RU24969 were significantly attenuated from $t=75$ min (Figure 5). Thus although the rate of onset and magnitude of the effect of RU24969 were not altered, the rate at which 5-HT levels returned to baseline was increased and the effects of RU24969 were short-lived in antidepressant-treated animals compared with control.

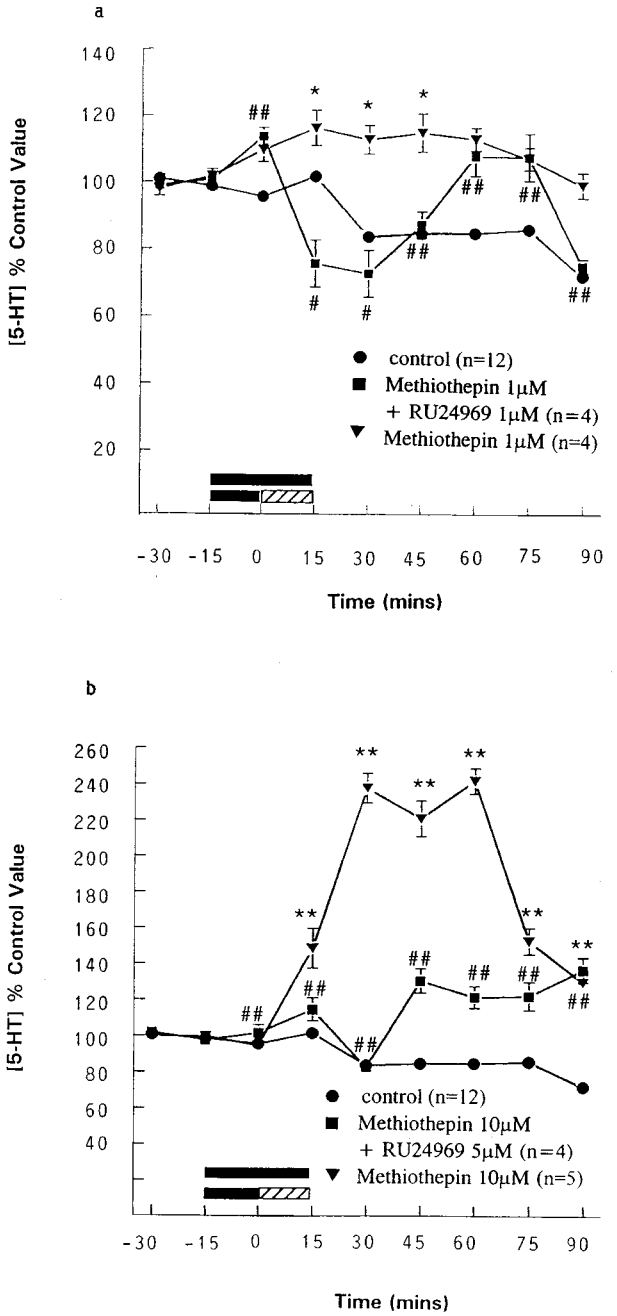

Figure 3 Effects of methiothepin infusion on the effects of RU24969 on 5-HT output in the anterior hypothalamus. Methiothepin was infused for 15 min and then either co-infused with RU24969 for a further 15 min (▨) or infused alone (■). (a) 1 μM methiothepin + 1 μM RU24969 ($n=4$) and 1 μM methiothepin ($n=4$), pooled control, $n=12$. *$P<0.05$ vs pooled control value, #$P<0.05$, ##$P<0.01$ vs 1 μM RU24969 (two-way ANOVA). (b) 10 μM methiothepin + 5 μM RU24969 ($n=4$) and 10 μM methiothepin ($n=5$), pooled control, $n=12$. **$P<0.01$ vs pooled control value, ##$P<0.01$ vs 5 μM RU24969 (two-way ANOVA). Data are expressed as a percentage of the concentration of 5-HT in the two dialysate samples taken immediately prior to any intervention (control value), as mean with s.e.mean indicated by vertical bars.

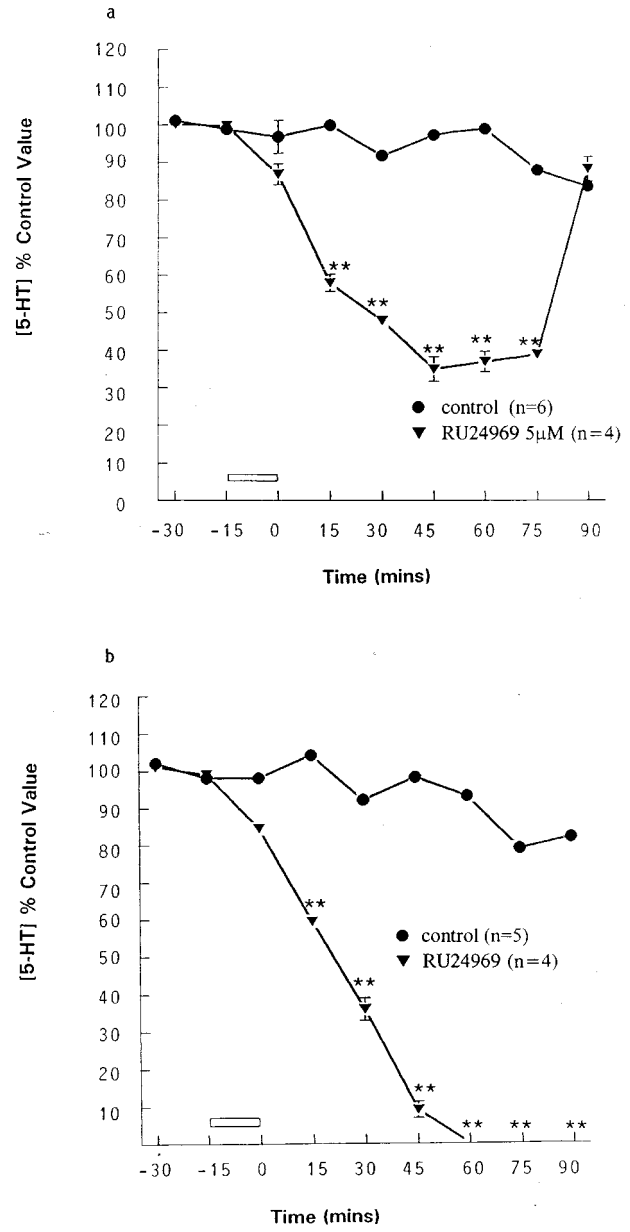

Figure 4 Effects of a 15 min infusion of 5 μM RU24969 at (a) mid-light and (b) end-light on 5-HT output in the anterior hypothalamus. (a) control ($n=6$) and 5 μM RU24969 ($n=4$). $^{**}P<0.01$ vs control value for mid-light (two-way ANOVA). (b) control ($n=5$) and 5 μM RU24969 ($n=4$). $^{**}P<0.01$ vs control value for end-light (two-way ANOVA). Data are expressed as a percentage of the concentration of 5-HT in the two dialysate samples taken immediately prior to any intervention (control value), as mean with the s.e.mean indicated by vertical bars.

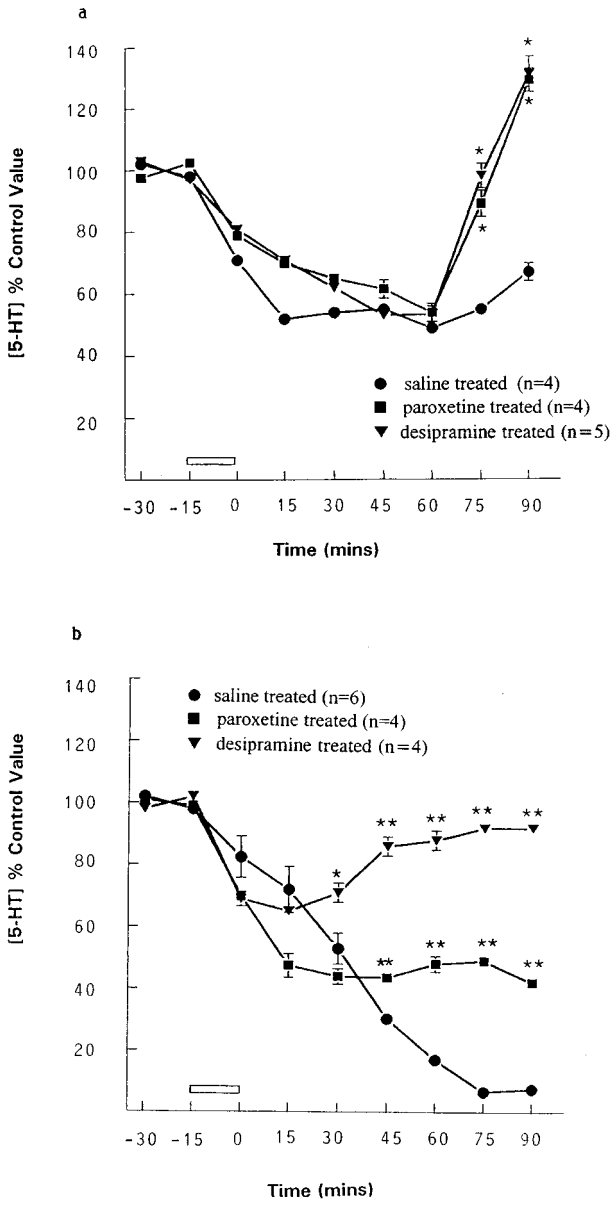

Figure 5 Rats were treated chronically with paroxetine hydrochloride (10 mg kg^{-1} i.p.), desipramine hydrochloride (10 mg kg^{-1} i.p.) or saline and the effect of a 15 min infusion of 5 μM RU24969 re-assessed at (a) mid-light and (b) end-light. (a) saline-treated + 5 μM RU24969 ($n=4$), paroxetine-treated + 5 μM RU24969 ($n=4$) and desipramine-treated + 5 μM RU24969 ($n=5$). $^{*}P<0.05$, vs saline-treated rats (two-way ANOVA). (b) saline-treated + 5 μM RU24969 ($n=6$), paroxetine-treated + 5 μM RU24969 ($n=4$) and desipramine-treated + 5 μM RU24969 ($n=4$). $^{*}P<0.05$, $^{**}P<0.01$ vs saline-treated animals (two-way ANOVA). Data are expressed as a percentage of the concentration of 5-HT in the two dialysate samples taken immediately prior to any intervention (control value), as mean with s.e.mean indicated by vertical bars.

Infusion of RU24969 (5 μM) *via* the probe at end-light decreased 5-HT output significantly in saline-treated rats, with a maximal inhibition of 99% (1.0% of control $\pm$1.8%, $n=4$, Figure 5). Chronic antidepressant treatment significantly attenuated this effect. Thus, the maximal inhibition of 5-HT output in desipramine-treated rats was 35% (65% of control $\pm$2.0% at $t=15$ min, $P<0.01$, $n=4$), and paroxetine treatment led to a maximum decrease in 5-HT release of 66.5% (43.5% of control $\pm$1.9% at $t=45$ min, $P<0.01$, $n=5$), (Figure 5). Therefore, at end-light the rate of onset and the magnitude of the response to RU24969 were significantly decreased after chronic antidepressant treatment (Figure 5).

Discussion and conclusions

Intracerebral microdialysis was used to investigate nerve terminal 5-HT$_{1B}$ autoreceptor function after chronic antidepressant treatment in the anterior hypothalamus of anaesthetized rats. The main finding of this study was that chronic antidepressant treatment attenuated the effect of RU24969 more markedly when infused locally at end-light, indicating that 5-HT$_{1B}$ autoreceptor function is attenuated in a time-of-day-dependant manner.

In order to confirm the origin of the 5-HT measured in the dialysate, two protocols were employed. Firstly, since exocytotic release is considered to be calcium dependent, calcium ions were removed from the aCSF for 1 h. This led to an immediate drop in 5-HT output which was reversed on the re-introduction of calcium ions into the aCSF. Secondly, aCSF with a high K^+ content (100 mM) was perfused for 15 min which produced a 3–4 fold increase in 5-HT efflux. These two findings confirm the neuronal origin of the 5-HT measured in dialysate and are consistent with results reported by other authors in the caudate-putamen (Kalen *et al*., 1988), ventromedial hypothalamus (Auerbach *et al*., 1989) and ventral hippocampus (Sharp *et al*., 1989).

The terminal 5-HT autoreceptor has been extensively examined and characterized as a 5-HT$_{1B}$ receptor (Martin & Sanders-Bush, 1982; Middlemiss, 1984; 1985; Martin & Marsden, 1986; Price *et al*., 1997; Schlicker *et al*., 1997; Trillat *et al*., 1997). We carried out a limited series of experiments to confirm that we were able to replicate the published findings. Thus, infusion of increasing concentrations of the 5-HT$_{1A/1B}$ agonist RU24969 (0.1–10 μM) led to a dose-dependent decrease in the level of 5-HT in the dialysate. The inhibitory effect of RU24969 on 5-HT release has been extensively demonstrated before (Sleight *et al*., 1989; Hjorth & Tao, 1991; Martin *et al*., 1992); however the dose-dependent effect has not been reported, albeit Martin *et al*. (1992) have shown that 0.1 μM had no effect on 5-HT output in the ventral hippocampus whereas 1 μM RU24969 decreased output by 47%. Since 12 μM RU24969 is well known to have almost equal affinity for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, to confirm that the overflow of 5-HT measured in dialysate was controlled by a 5-HT$_{1B}$ autoreceptor, and to ensure that any effect of RU24969 was mediated by the stimulation of a 5-HT$_{1B}$ receptor and not a 5-HT$_{1A}$ receptor, the selective 5-HT$_{1A/7}$ receptor agonist, 8-OH-DPAT, was infused *via* the probe. 8-OH-DPAT (1 μM) had no effect on 5-HT output. Further corroboration could have been sought by examining the effect of RU24969 following treatment with WAY 100635; however it seems reasonable to conclude that hypothalamic 5-HT$_{1A}$ and 5-HT$_7$ receptors do not affect 5-HT overflow in the anterior hypothalamus and the effects of RU24969 reported here were produced solely by stimulation of a 5-HT$_{1B}$ receptor.

Pre-infusion of methiothepin (10 μM) blocked the effects of RU24969 (5 μM) as has been described previously (O'Connor & Kruk, 1991; Martin *et al*., 1992). Infusion of methiothepin alone (1 μM and 10 μM) increased the overflow of 5-HT as has also been reported in hypothalamic slices *in vitro* (Pettibone & Pflueger, 1984) and *in vivo* (Baumann & Waldmeier, 1984; Martin & Marsden, 1986; O'Connor & Kruk, 1991; Roberts *et al*., 1997). Methiothepin is a 5-HT receptor antagonist which shows some selectivity for 5-HT$_1$ receptors (Hibert & Middlemiss, 1986), however it is not entirely selective for 5-HT receptors. Methiothepin is also an antagonist at α_2-adrenoceptors which are known to be present on 5-HT terminals and to have an inhibitory effect on 5-HT release *in vivo* (Tao & Hjorth, 1992) which is tonic (Marsden & Martin, 1986; Mongeau *et al*., 1993). The increased 5-HT output after methiothepin was concentration dependent (the increase after 1 μM was 15% and after 10 μM 142%). Thus the increased output of 5-HT may be attributable to inhibition of the autoinhibitory tone of both the 5-HT$_{1B}$ autoreceptor as well as the α_2-adreno-heteroreceptor. However, since methiothepin (1 μM) attenuated the effects of RU24969 whilst having negligible effects on 5-HT output itself, it is reasonable to conclude that, in view of the data obtained here and extensively reported in the literature (see above discussion), RU24969 exerts its effects on 5-HT release in the rat *via* the 5-HT$_{1B}$ autoreceptor.

RU24969 administration caused a quantitatively greater inhibition of 5-HT output at end-light compared to mid-light. The greater effect of infusion of 5 μM RU24969 at end-light compared to mid-light could be due to a difference in the amount of 5-HT released and therefore receptor occupation by the endogenous ligand, or the sensitivity/number of autoreceptors at the two time points. A significant 24 h variation in the number of 5-HT$_{1B}$ binding sites has been demonstrated, but only in the cortex (Akiyoshi *et al*., 1989). The number of binding sites at mid-light was significantly higher than at end-light which does not correlate with the findings presented here which suggest that 5-HT$_{1B}$ receptor function is greatest at end-light. The differences could be because the receptor binding studies of Akiyoshi *et al*. (1989) measured both presynaptic and postsynaptic receptors. Alternatively, the cortical rhythm may be different from that in the hypothalamus. mRNA levels for the 5-HT$_{1B}$ receptor in the suprachiasmatic nuclei (SCN) of the hypothalamus have not been found to vary over 24 h (Roca *et al*., 1993), when measured at four equally spaced time points in the light-dark cycle. However since mRNA is not normally found in nerve terminals, in these experiments it can be taken as a measure of postsynaptic receptors in the SCN; this does not therefore preclude a rhythm in presynaptic receptors. Alternatively, there could be circadian rhythms in receptor-effector coupling, adenylate cyclase activity or the intracellular level of cyclic AMP. Prosser & Gillette (1991) have described a rhythm in phosphodiesterase activity in rat SCN, which is responsible for the circadian rhythm in cyclic AMP and noradrenaline observed.

In the experiments reported here, rats were treated chronically with two different types of antidepressant; *viz*. desipramine, a selective noradrenaline uptake inhibitor and paroxetine, a selective 5-HT uptake inhibitor (SSRI); no significant effects on basal 5-HT efflux were observed at either time point.

The effects of antidepressant treatment on 5-HT$_{1B}$ autoreceptor function at mid-light will be considered first because it is at this time point that most other studies assessing neurotransmitter receptor binding and function are carried out. When the effect of RU24969 (5 μM) was re-assessed after chronic antidepressant treatment at mid-light, there was no significant difference in the initial effect of RU24969 infusion between antidepressant- or saline-treated animals. Neither desipramine nor paroxetine significantly altered the maximal response of the autoreceptor to RU24969. However, there was a significant increase in 5-HT output over base line at the end of the experiment indicating that the effects of RU24969 were terminated more quickly in antidepressant pre-treated animals. These results are in agreement with the only comparable published study by Sleight *et al*. (1989), assuming that their experiments were performed at or near the middle of the light phase. These workers found that chronic treatment with either amitriptyline or MDL72394 (a MAOI) had no effect on the response to RU24969 (10 mg kg^{-1} i.p.) in the frontal cortex of the anaesthetized rat. Other *in vivo* studies, using microiontophoresis, have indirectly demonstrated autoreceptor down-regulation in the hippocampus after chronic fluoxetine or citalopram but not clorgyline treatment (Chaput *et al*., 1986; Blier *et al*., 1988). The results of studies performed *in vitro* are equivocal, reporting both a decrease in sensitivity after treatment with MAOI, selective 5-HT uptake inhibitors or non-selective 5-HT and noradrenaline uptake inhibitors (Maura & Raiteri, 1984; Moret & Briley, 1990) and a decrease (Johanning *et al*., 1992) or no change in the number of 5-HT$_{1B}$

binding sites (Montero *et al*., 1991) after treatment with a SSRI.

By contrast, at end-light prolonged antidepressant treatment significantly attenuated the effects of RU24969; desipramine appeared to down-regulate 5-HT$_{1B}$ autoreceptor function more effectively than paroxetine. This down-regulation of 5-HT$_{1B}$ autoreceptor function following chronic desipramine treatment might result from a desensitization of terminal α_2-adrenoceptors as a result of an increase in noradrenaline around the 5-HT nerve terminal following blockade of noradrenaline reuptake. Down-regulation of the α_2-adrenoceptor would decrease the feedback inhibition of this receptor on 5-HT release, the biophase level of 5-HT would then rise resulting in down-regulation of the 5-HT$_{1B}$ autoreceptor. Treatment with paroxetine would have a more direct effect. Blockade of 5-HT reuptake would directly increase 5-HT levels in the synaptic cleft thus allowing desensitization of the autoreceptor. Since this is the first observation that antidepressant drugs decrease 5-HT$_{1B}$ autoreceptor function *in vivo* it is pertinent to consider the possible mechanisms involved. The down-regulation may be attributable to changes in autoreceptor and/or α_2-heteroadrenoceptor expression, expression of G proteins (Lesch *et al*., 1991), uncoupling of the receptor-effector mechanism (Okada *et al*., 1988; Rasenick & Wang, 1988) or changes in intracellular signal transduction pathways (Nestler *et al*., 1989; Perez *et al*., 1989).

Although the reason for the greater effect of desipramine is unclear, as outlined above the down-regulation in the α_2-heteroadrenoreceptor may increase the terminal biophase concentration of 5-HT and perhaps expose the terminal autoreceptor to a greater level of 5-HT at end-light compared to mid-light, thus leading to greater down-regulation at this time point.

In conclusion, this study has demonstrated a significant difference in the function of the terminal 5-HT$_{1B}$ autoreceptor at two time points during the light phase of the light–dark cycle *in vivo*. More significantly, however, the data show that autoreceptor function is attenuated following prolonged antidepressant treatment and that this effect is time-of-day-dependent. This finding may explain why a down-regulation of 5-HT autoreceptor function has not been consistently reported in previous studies.

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