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Modulation of sibutramine-induced increases in extracellular noradrenaline concentration in rat frontal cortex and hypothalamus by α_2-adrenoceptors

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1 The effects of sibutramine (0.25–10 mg kg^{-1} i.p.) on extracellular noradrenaline concentration in the frontal cortex and hypothalamus of freely-moving rats were investigated using microdialysis. The role of presynaptic α_2-adrenoceptors in modulating the effects of sibutramine in these brain areas was also determined.

2 Sibutramine induced an increase in extracellular noradrenaline concentration, the magnitude of which paralleled dose, in both brain areas. In the cortex, this increase was gradual and sustained, whereas in the hypothalamus it was more rapid and of shorter duration.

3 In both the cortex and hypothalamus, pretreatment of rats with the α_2-adrenoceptor antagonist RX821002 (3 mg kg^{-1} i.p.) potentiated increases in the accumulation of extracellular noradrenaline induced by sibutramine (10 mg kg^{-1} i.p.), by 7 and 10 fold respectively. RX821002 also reduced the latency of sibutramine to reach its maximum effect in the cortex, but not in the hypothalamus.

4 Infusion of RX821002 (1 μM) *via* the probe increased the accumulation of extracellular noradrenaline induced by sibutramine (10 mg kg^{-1} i.p.) in both brain areas. In the hypothalamus, the effects of RX821002 on the accumulation of noradrenaline induced by sibutramine were 2 fold greater than those in the cortex.

5 These findings support evidence that sibutramine inhibits the reuptake of noradrenaline *in vivo*, but that the accumulation of extracellular noradrenaline is limited by noradrenergic activation of presynaptic α_2-adrenoceptors. Furthermore, the data suggest that terminal α_2-adrenoceptors in the hypothalamus exert a greater inhibitory effect over the control of extracellular noradrenaline accumulation than do those in the cortex.

Keywords: Sibutramine; noradrenaline; α_2-adrenoceptors; RX821002; frontal cortex; hypothalamus; *in vivo* microdialysis

Abbreviations: aCSF, artificial cerebrospinal fluid; 5-HT, 5-hydroxytryptamine; PVN, paraventricular nucleus

Introduction

Sibutramine (BTS 54 524; N-(1-[1-(4-chlorophenyl)cyclobutyl] - 3 - methylbutyl) - 3 - N, N-dimethylamine hydrochloride monohydrate) causes weight loss in rats by decreasing food intake through the enhancement of satiety (Halford *et al*., 1995). It also increases energy expenditure in rats through the enhancement of thermogenesis (Stock, 1997). Both these anti-obesity effects of sibutramine result from a synergistic mechanism involving 5-hydroxytryptamine (5-HT) and noradrenaline reuptake inhibition in the central nervous system (Heal & Cheetham, 1997; Jackson *et al*., 1997a,b; Stock, 1997). Following administration to either animals or humans, sibutramine is rapidly metabolized to the secondary amine, Metabolite 1 (BTS 54 354; *N*-{1-[1-(chlorophenyl)cylcobutyl]-*N*-methylamine hydrochloride) and then to the primary amine, Metabolite 2 (BTS 54 505; 1-[1-(4-chlorophenyl)cylcobutyl]-*3*-methylbutylamine hydrochloride) (Heal *et al*., 1998). These active metabolites are potent inhibitors of both noradrenaline and 5-HT uptake *in vitro* (Cheetham *et al*., 1993; 1996) and predominantly mediate the pharmacological effects of sibutramine *in vivo* (Luscombe *et al*., 1989). In keeping with these findings, this drug has been shown to be a potent inhibitor of 5-HT and noradrenaline reuptake *in vivo* (Luscombe *et al*., 1989; Gundlah *et al*., 1997; Wortley *et al*., 1999).

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Previous studies in the frontal cortex of anaesthetized rats have shown that the increase in extracellular noradrenaline concentration induced by sibutramine is gradual and sustained, and that this effect is mediated by noradrenergic activation of α_2-adrenoceptors (Wortley *et al*., 1999). These adrenergic autoreceptors are located on the cell bodies and terminals of noradrenergic neurones, where they act to depress neuronal firing and inhibit exocytotic release of noradrenaline, respectively (Cedarbaum & Aghajanian, 1976; Langer, 1977). Microdialysis studies using selective α_2-adrenoceptor agonists and antagonists have demonstrated that these receptors regulate extracellular concentrations of noradrenaline in the cortex (L'Heureux *et al*., 1986) and that both somatodendritic and terminal α_2-adrenoceptors contribute to this control (Dennis *et al*., 1987; van Veldhuizen *et al*., 1993; Dalley & Stanford, 1995; Mateo *et al*., 1998).

Although many *in vivo* studies have investigated the role of α_2-adrenoceptors in the control of noradrenaline release in the cortex, less is known about the actions of α_2-adrenoceptors in the hypothalamus. This latter brain area is important in the regulation of appetite (Grossman, 1975), and noradrenergic transmission within the hypothalamus can markedly influence feeding behaviour (Grossman, 1960; Leibowitz, 1976; Wellman, 1992). Although binding studies demonstrate the presence of α_2-adrenoceptors in the hypothalamus (U'Prichard *et al*., 1977; Stanford *et al*., 1983; Heal *et al*., 1993), their influence in this area cannot be assumed to be the same as that

in the cortex for a number of reasons. Firstly, the cortex and hypothalamus differ in respect of the source of their noradrenergic innervation: the cortex receives noradrenergic neurones exclusively from the locus coeruleus, while the hypothalamus is primarily innervated by noradrenergic neurones with cell bodies in the lateral tegmental nuclei (Holets, 1990); evidence suggests that there could be functional differences between these two groups of neurones (Zaczek *et al*., 1990). Secondly, studies using DSP-4 have shown that noradrenergic neurones in the hypothalamus have a greater density of presynaptic α_2-adrenoceptors than those in cortical regions (Heal *et al*., 1993).

The first aim of this study was to establish the effects of sibutramine on extracellular noradrenaline concentration in the hypothalamus using *in vivo* microdialysis, and to compare these effects with those in the frontal cortex. Drug-induced changes in extracellular noradrenaline concentration were monitored in the region of the paraventricular nucleus (PVN) because changes in noradrenergic activity in this specific area of the hypothalamus show the greatest influence on food intake compared with other brain regions (Matthews *et al*., 1977; Leibowitz, 1978; Wellman, 1992). Increased noradrenergic activity in the hypothalamus has been associated with both an increase and decrease in feeding behaviour; activation of α_1-adrenoceptors with phenylpropanolamine induces a suppression of food intake, whereas activation of α_2-adrenoceptors with clonidine has a biphasic effect, with low doses facilitating and high doses inhibiting food intake (Wellman, 1992).

The second aim of the study was to compare the contribution of α_2-adrenoceptors to the control of noradrenaline release in the frontal cortex and hypothalamus. This involved firstly, establishing the effects of sibutramine on extracellular noradrenaline concentrations in the cortex or hypothalamus in rats pretreated systemically with the selective α_2-adrenoceptor antagonist RX821002 (Langin *et al*., 1990) or atipamezole (Scheinin *et al*., 1988). The systemic administration of an α_2-adrenoceptor antagonist affects all populations of α_2-adrenoceptors, including those at the level of the cell bodies. To evaluate the contribution of terminal α_2-adrenoceptors to noradrenaline release in the cortex or hypothalamus, further experiments investigated the effects of RX821002 when this was administered locally through the dialysis probe following sibutramine administration.

Methods

Animals

All procedures complied with the Animals (Scientific Procedures) Act 1986. Male Sprague-Dawley rats (250–320 g) were obtained from the colony bred at University College London and used throughout this study. Rats were housed in groups of four with a light-dark cycle of 12 h (lights on at 08.00 h) and had free access to food and water.

Surgical procedures

Dialysis probes were constructed with a semipermeable membrane (i.d. 200 μm, o.d. 300 μm, mol. wt. cut off 20 kD; Filtral 12, Hospal Industrie, France) essentially as described by Sandberg *et al*. (1986). Probes had a dialysis zone of 5 or 1.5 mm for the frontal cortex and the region of the paraventricular nucleus (PVN), respectively. Rats were placed in an induction chamber and anaesthesia induced by inhalation of halothane (5% halothane mixed in 95% oxygen and 5% CO_2 at 2 l min^{-1}). Rats were then placed in a Kopf stereotaxic frame where anaesthesia was maintained *via* a face mask (2% halothane mixed in 95% oxygen and 5% CO_2 at 1 l min^{-1}). The core body temperature was maintained at 37°C throughout the surgery using a homeothermic heating blanket and rectal probe (Harvard Instruments). The skull was exposed to reveal bregma and, following craniotomy, a microdialysis probe primed with artificial cerebrospinal fluid ('aCSF' mM: NaCl 140; KCl 3; $CaCl_2$ 1.2; $MgCl_2$ 1.0; Na_2HPO_4 1.2; NaH_2PO_4 0.27; glucose 7.2; pH 6.8) was lowered vertically into the frontal cortex (mm: AP +3.5, L −1.5, relative to bregma and DV −5.0, relative to dura) or the region of the paraventricular nucleus of the hypothalamus (mm: AP −1.8, L −0.5, DV −9.2) according to the atlas of Paxinos & Watson (1986). Probes were secured with dental cement and anchored by two wood screws inserted into the skull. The rats were allowed to recover from anaesthesia in an incubation chamber and then transferred to individual cages. Experiments were carried out the next day.

Microdialysis

The dialysis probe was perfused with aCSF at a rate of 1 μl min^{-1} for at least 2 h before samples were collected at 20 min intervals. Each series of experiments investigated drug effects on extracellular noradrenaline concentrations in both frontal cortex and hypothalamus. The first series of experiments began with the collection of four baseline ('basal') samples; this was followed by administration of sibutramine (0.25, 1 or 10 mg kg^{-1} i.p.) or vehicle, after which samples were collected for a further 4 h. The doses of sibutramine were chosen from previous studies showing that, over this concentration-range, sibutramine has dose-dependent effects on food-intake as well as extracellular 5-HT and noradrenaline accumulation (Jackson *et al*., 1997b; Gundlah *et al*., 1997; Wortley *et al*., 1999).

In a second series of experiments, rats were divided into three groups. After the collection of four basal samples, one group of rats received 3 mg kg^{-1} (i.p.) of the selective α_2-adrenoceptor antagonist, RX821002, and 2 h later received sibutramine (10 mg kg^{-1}) or vehicle. RX821002 is currently one of the most selective α_2-adrenoceptor antagonists available and was administered at 3 mg kg^{-1} based on a previous study showing that this drug effectively blocks presynaptic α_2-adrenoceptors at this concentration (Wortley *et al*., 1999). A second group of rats received 1 mg kg^{-1} (i.p.) of the selective α_2-antagonist, atipamezole, following the collection of four basal samples and 2 h later received sibutramine (10 mg kg^{-1}). Atipamezole is also one of the most highly selective α_2-adrenoceptor antagonists available and was administered at 1 mg kg^{-1} i.p. because this induced an increase in baseline noradrenaline concentration which was comparable with 3 mg kg^{-1} RX821002. A third group of rats received vehicle following collection of four basal samples and 2 h later received sibutramine (10 mg kg^{-1}). In all groups of rats, samples were collected for a further 3 h following the second injection. A time period of 2 h was left between each injection to allow a clear differentiation between the increase in extracellular noradrenaline concentration induced by each drug administration.

A third series of experiments examined the effects of local administration of RX821002 into the frontal cortex or hypothalamus on the increases in extracellular noradrenaline

concentration induced by 10 mg kg^{-1} sibutramine. Following the collection of four basal samples, rats were injected with either sibutramine (10 mg kg^{-1}) or vehicle and, 80 min later, RX821002 was administered *via* the dialysis probe at a concentration of 1 μM. A time period of 80 min was left between the injection of sibutramine and the administration of RX821002 to allow a clear differentiation between the initial increase in extracellular noradrenaline induced by sibutramine and the enhanced increase induced by α_2-adrenoceptor blockade.

HPLC analysis of dialysates

The noradrenaline content of the dialysate samples was analysed using reverse-phase high pressure liquid chromatography coupled to an electrochemical detector. Solutes were separated at room temperature using a Hypersil ODS 5 μm column (250 × 4.6 mm) protected by an Aquapore guard column (30 mm × 4.6 mm; Applied Biosystems). The mobile phase comprised (mM): octane sulphonic acid 2; sodium dihydrogen orthophosphate 83; EDTA 0.85; methanol 12%; and was adjusted to pH 4 with orthophosphoric acid. This was filtered, degassed and recycled at 1.3 ml min^{-1}. Noradrenaline was detected using a high performance analytical cell (model 5014A; ESA) controlled by a Coulochem detector (ESA, model 5100A). Potentials were set in REDOX mode (detector 1: −180 mV: detector 2: +180 mV). The mobile phase was conditioned using a pre-injection guard cell set at +350 mV. Chromatograms were relayed to a Spectra-Physics Chromjet integrator. Dialysate noradrenaline concentration was determined using peak height compared to an external standard. Concentrations are expressed as fmol 20 min^{-1} and were not corrected for probe recovery.

Drugs and reagents

Drugs were either dissolved in 0.9% saline and administered intraperitoneally (i.p.) at a volume of 2 ml kg^{-1}, or dissolved in aCSF and administered *via* the dialysis probe. The following drugs were used: atipamezole hydrochloride (Farmos), 2-[2-(2-methoxy-1,4-benzodioxanyl)] imidazoline hydrochloride (RX821002; Research Biochemicals Incorporated), sibutramine hydrochloride (Knoll Pharmaceuticals Research). Halothane was obtained from Zeneca. Reagents were either AnalaR or HPLC grade (BDH Ltd or Sigma).

Statistical analysis

Changes in the noradrenaline content of dialysis samples after drug injection were tested for statistical significance using the ANOVA repeated measures facility on SPSS PC.$^+$ Analysis was carried out on orthonormalized raw data (i.e. on orthogonal contrasts: Norusis, 1988) with 'time' as the 'within-subjects' factor. Averaged F tests were used to assess the statistical significance of any differences in noradrenaline concentration. To compare the effects of different drug treatments, split-plot ANOVA was used with 'time' as the 'within-subjects factor' and 'drug treatment' as the 'between-subjects' factor. If statistically significant main effects of drug treatment were exposed, data were split into time-matched bins of consecutive samples and ANOVA used to compare the effects of different treatments within each bin. Finally, RX821002-induced changes in the latency to reach peak dialysate noradrenaline concentration were assessed: the mean latency to reach the peak drug effect for individual rats was determined and one-way ANOVA used to compare the effects of different treatment groups. The criterion for statistical significance was set at $P<0.05$.

Results

Effects of sibutramine on dialysate noradrenaline concentration from the frontal cortex

The mean noradrenaline content of the basal samples was 26.2 ± 0.6 fmol 20 min^{-1} (pooled data from all experiments carried out in the frontal cortex). There was no change in dialysate noradrenaline concentration in rats injected with saline vehicle (Figure 1), but there was a gradual and sustained increase following administration of sibutramine at 0.25, 1 or 10 mg kg^{-1} (Figure 1).

The magnitude of the increase in noradrenaline induced by sibutramine was dose-dependent. 0.25 mg kg^{-1} sibutramine failed to increase dialysate noradrenaline concentrations significantly. However, sibutramine (1 or 10 mg kg^{-1}) induced a significant increase in noradrenaline concentration within 40 min post-injection (1 mg kg^{-1}: $F=4.24$; *d.f.* 2,8; $P=0.05$; 10 mg kg^{-1}: $F=7.56$; *d.f.* 2,8; $P=0.01$). The largest increase was induced by the largest dose (10 mg kg^{-1}: maximum of 518%, *c.f.* basals) and occurred at 140 min post-injection. The maximum increase after 1 mg kg^{-1} sibutramine was 291% and occurred at 80 min post-injection.

Effects of systemic administration of RX821002 or atipamezole on sibutramine-induced increases in dialysate noradrenaline concentration from the frontal cortex

RX812002 (3 mg kg^{-1} i.p.) induced a 2 fold increase in dialysate noradrenaline concentration (Figure 2). The increase in noradrenaline concentration was greater than that induced by saline injection (effect of treatment: $F=25.3$; *d.f.* 1,8; $P<0.01$).

At 120 min following the injection of RX821002, when dialysate noradrenaline concentration had become stable again, an i.p. injection of saline had no further effect on

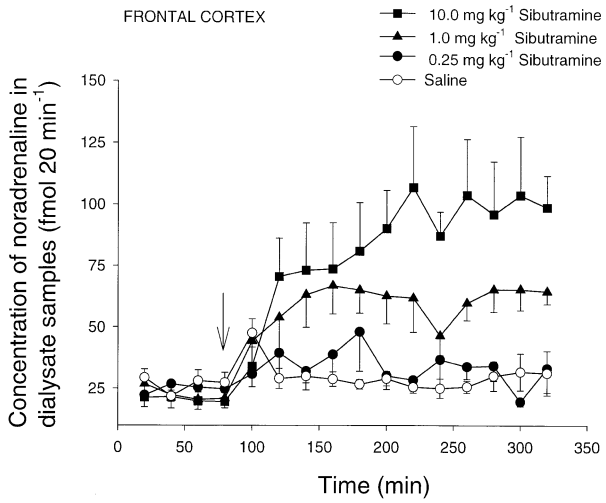

Figure 1 The effects of sibutramine (0.25, 1 or 10 mg kg^{-1} i.p.) on the concentration of noradrenaline in dialysate samples from the frontal cortex. Arrow represents drug administration. Data expressed as mean ± s.e.mean noradrenaline concentration (fmol 20 min^{-1}; $n=4-6$ for each group).

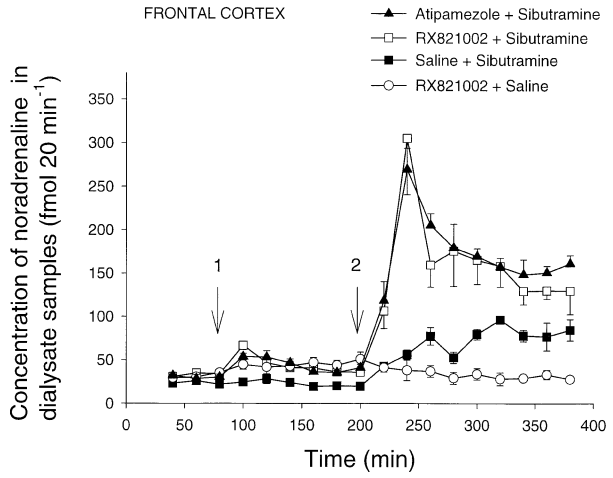

Figure 2 The effect of sibutramine (10 mg kg^{-1} i.p.) on the concentration of noradrenaline in dialysate samples from the cortex of rats pretreated with either RX821002 (3 mg kg^{-1} i.p.) or atipamezole (1 mg kg^{-1} i.p.). Arrow 1 represents administration of RX821002, atipamezole or vehicle. Arrow 2 represents administration of sibutramine or vehicle. Data expressed as mean$\pm$s.e.mean noradrenaline concentration (fmol 20 min^{-1}; $n=4-6$ for each group).

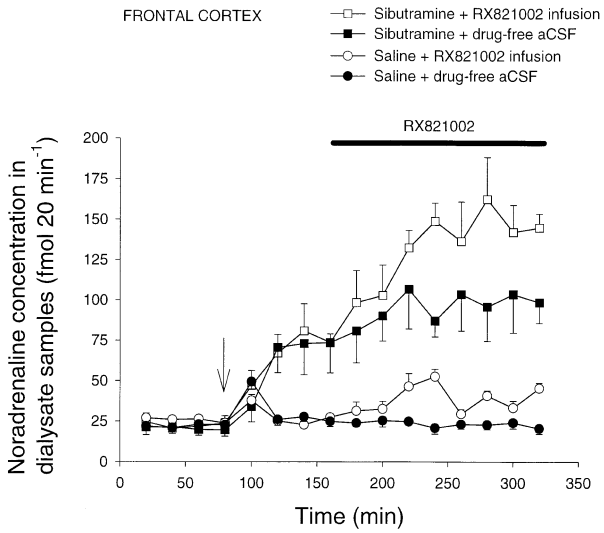

Figure 3 The effect of local administration of RX821002 (1 μM) on basal (circles), and sibutramine-induced (10 mg kg^{-1} i.p. squares) increases in dialysate noradrenaline concentration from the frontal cortex. Arrow represents administration of sibutramine or vehicle. Bar indicates the period of RX821002 perfusion *via* the dialysis probe. Open symbols represent data from rats perfused with RX821002. Closed symbols represent rats perfused with drug-free aCSF. Data expressed as mean$\pm$s.e.mean noradrenaline concentration (fmol 20 min^{-1}; $n=5-6$ for each group).

dialysate noradrenaline concentration (Figure 2). However, an i.p. injection of sibutramine at this point (10 mg kg^{-1}), caused a rapid increase in dialysate noradrenaline concentration. The increase was statistically significant at 20 min ($F=9.8$; *d.f.* 1, 5; $P=0.03$) and reached a maximum of 900% (*c.f.* basal samples) within 40 min. Following this, dialysate noradrenaline concentration decreased to a plateau representing an approximately 4.5 fold increase in noradrenaline (*c.f.* basals). In contrast, when sibutramine (10 mg kg^{-1} i.p.) was administered 120 min after an i.p. injection of saline, there was a gradual increase in dialysate noradrenaline concentration and the maximum (438%) was not reached until 120 min post-injection (Figure 2). At all times following the injection of sibutramine, dialysate noradrenaline concentrations in RX821002-pretreated rats were significantly greater than in saline-pretreated rats (last hour: $F=7.95$; *d.f.* 1,7; $P=0.03$). Furthermore, the latency to reach maximum noradrenaline concentration was less in rats pretreated with RX821002 than with saline ($F=10.67$; *d.f.* 1, 7; $P=0.02$).

Atipamezole (1 mg kg^{-1} i.p.) induced an increase in dialysate noradrenaline concentration which was similar in magnitude and time-course to that seen with RX821002 (3 mg kg^{-1} i.p.) (Figure 2). An injection of sibutramine (10 mg kg^{-1} i.p.) 2 h after atipamezole also induced an increase in dialysate noradrenaline concentration similar to that seen in rats pretreated with 3 mg kg^{-1} RX821002 (effect of pretreatment: $F=0.16$; *d.f.* 1,4; $P=0.70$) (Figure 2).

Effects of local administration of RX821002 on sibutramine-induced increases in dialysate noradrenaline concentration from the frontal cortex

Administration of RX821002 (1 μM) *via* the probe 80 min after saline injection (2 ml kg^{-1} i.p.), increased basal noradrenaline concentration compared with that of rats infused with drug-free aCSF ($F=22.78$; *d.f.*, 1,6; $P<0.01$) (Figure 3). RX821002 administered 80 min after sibutramine (10 mg kg^{-1} i.p.), also increased dialysate noradrenaline concentration compared with rats infused with drug-free aCSF ($F=6.87$; *d.f.* 1,7; $P=0.03$) (Figure 3). Within 80 min of the start of RX821002 infusion, the increase in noradrenaline concentration caused by sibutramine was approximately 1.5 fold greater than the dialysate noradrenaline concentration induced by sibutramine alone.

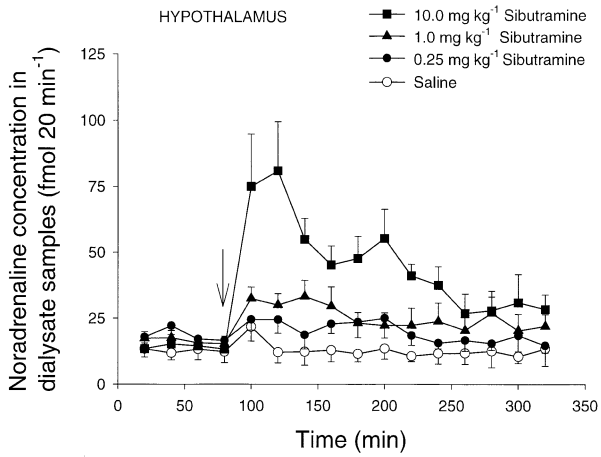

Figure 4 The effects of sibutramine (0.25, 1 or 10 mg kg^{-1} i.p.) on the concentration of noradrenaline in dialysate samples from the hypothalamus. Arrow represents drug administration. Data expressed as mean$\pm$s.e.mean noradrenaline concentration (fmol 20 min^{-1}; $n=4-8$ for each group.

Effects of sibutramine on dialysate noradrenaline concentration from hypothalamus

The mean noradrenaline content of the basal samples was 13.9$\pm$0.6 fmol 20 min^{-1} (pooled data from all experiments carried out in the hypothalamus). There was no change in dialysate noradrenaline concentration in rats injected with saline (Figure 4), but dialysate noradrenaline concentration

increased following administration of sibutramine at 0.25, 1 or 10 mg kg^{-1}.

The magnitude of the increase in noradrenaline concentration induced by sibutramine was dose-dependent. A dose of 0.25 mg kg^{-1} failed to significantly increase noradrenaline concentration. Sibutramine (1 and 10 mg kg^{-1}) increased dialysate noradrenaline within 20 min post-injection (1 mg kg^{-1}: $F=26.55$; *d.f.* 11,6; $P<0.01$; 10 mg kg^{-1}: $F=12.34$; *d.f.* 1,7; $P=0.01$). The largest increase was induced by the largest dose (10 mg kg^{-1}: maximum of 610%, *c.f.* basals) and occurred 40 min post-injection. The maximum increase after 1 mg kg^{-1} sibutramine was 217% and occurred at 60 min post-injection.

Effects of systemic administration of RX821002 or atipamezole on sibutramine-induced increases in dialysate noradrenaline concentration from the hypothalamus

RX821002 (3 mg kg^{-1} i.p.) induced a 2 fold increase in dialysate noradrenaline concentration (Figure 5). This increase in noradrenaline was greater than the increase induced by saline injection ($F=6.45$; *d.f.* 1,5; $P=0.05$).

At 120 min following the injection of RX821002, when dialysate noradrenaline concentration had become stable again, an i.p. injection of saline did not significantly affect dialysate noradrenaline concentration (Figure 5). In contrast, an i.p. injection of sibutramine at this point caused a rapid increase in dialysate noradrenaline concentration, which reached a maximum of 750% (*c.f.* basal samples) at 20 min ($F=44.67$; *d.f.* 3,6; $P<0.01$). Following this, dialysate noradrenaline concentration declined steadily to an approximately 3 fold increase in noradrenaline (*c.f.* basals), 80 min later. Sibutramine (10 mg kg^{-1} i.p.) administered 120 min after an i.p. injection of saline, induced a maximum increase in noradrenaline concentration of approximately 410% at 40 min post-injection ($F=11.05$; *d.f.* 3,15; $P<0.01$). Although this was a smaller initial increase in noradrenaline compared with that induced by sibutramine in RX821002-pretreated rats ($F=115.09$; *d.f.* 1,7; $P<0.01$), there was no difference in noradrenaline concentration between the two treatment groups 2 h after sibutramine injection. There was also no difference in the latency to reach maximum noradrenaline concentration in rats pretreated with RX821002 or saline.

Atipamezole (1 mg kg^{-1} i.p.) induced an approximately 3 fold increase in dialysate noradrenaline concentration (Figure 5). An injection of sibutramine 2 h after atipamezole induced an increase in dialysate noradrenaline similar to that observed in rats pretreated with 3 mg kg^{-1} RX821002 ($F=0.34$; *d.f.* 1,5; $P=0.59$) (Figure 5).

Effects of local administration of RX821002 on sibutramine-induced increases in dialysate noradrenaline concentration from the hypothalamus

Administration of RX821002 (1 μM) *via* the probe 80 min after saline (2 ml kg^{-1} i.p.), increased basal noradrenaline concentration compared with samples from rats infused with drug-free aCSF ($F=22.78$; *d.f.* 1,6; $P<0.01$) (Figure 6). RX821002 administered *via* the probe, 80 min after sibutramine (10 mg kg^{-1} i.p.), also increased dialysate noradrenaline concentration compared with rats infused with drug-free aCSF ($F=6.9$; *d.f.* 1,6.; $P=0.04$) (Figure 6). Within 80 min of the start of the RX821002 infusion, and for the rest of the experiment, the increase in noradrenaline concentration caused by sibutramine was approximately 3 fold greater than the increase induced by sibutramine alone.

Discussion

The anti-obesity agent, sibutramine, causes weight loss in rats through a dual action to decrease food intake (Halford *et al.*, 1995; Jackson *et al.*, 1997a,b) and to increase resting energy expenditure (Stock, 1997). Evidence suggests that the inhibition of neuronal reuptake of noradrenaline in the central nervous system has an integral role in the mediation of both

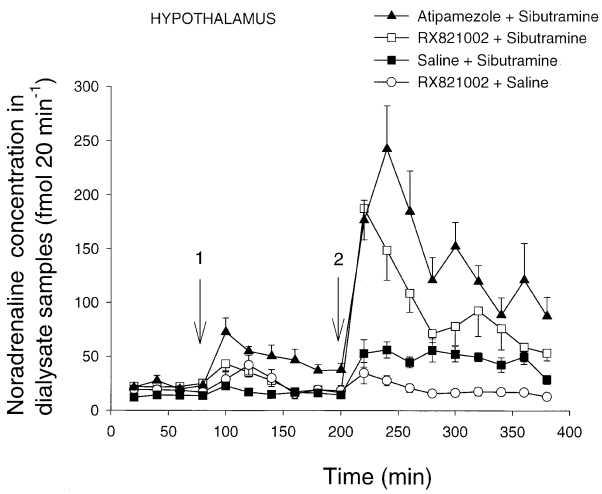

Figure 5 The effect of sibutramine (10 mg kg^{-1} i.p.) on the concentration of noradrenaline in dialysate samples from the hypothalamus of rats pretreated with either RX821002 (3 mg kg^{-1} i.p.) or atipamezole (1 mg kg^{-1} i.p.). Arrow 1 represents administration of RX821002, atipamezole or vehicle. Arrow 2 represents administration of sibutramine or vehicle. Data expressed as mean$\pm$ s.e.mean noradrenaline concentration (fmol 20 min^{-1}; $n=4-7$ for each group).

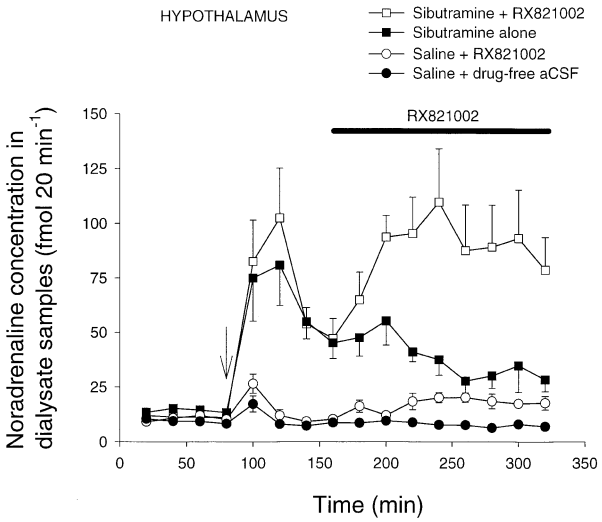

Figure 6 The effect of local administration of RX821002 (1 μM) on basal (circles), and sibutramine-induced (10 mg kg^{-1} i.p., squares) increases in dialysate noradrenaline concentration from the hypothalamus. Arrow represents administration of sibutramine or vehicle. Bar indicates the period of RX821002 perfusion *via* the dialysis probe. Open symbols represent data from rats perfused with RX821002. Closed symbols represent rats perfused with drug-free aCSF. Data expressed as mean$\pm$s.e.mean noradrenaline concentration (fmol 20 min^{-1}; $n=5-6$ for each group).

responses (Jackson *et al*., 1997a,b; Heal & Cheetham, 1997). Consistent with these findings, sibutramine increases extracellular noradrenaline concentration in the cortex of anaesthetized rats, the magnitude of which is restricted by noradrenergic activation of α_2-adrenoceptors (Wortley *et al*., 1999). Since noradrenergic transmission in the hypothalamus, and in particular the PVN, has been more strongly implicated in the control of feeding than the cortex (Leibowitz, 1976; 1978; Wellman, 1992), this study investigated the actions of sibutramine in the hypothalamus and compared these effects with those observed in the frontal cortex.

In agreement with our earlier study, the present results show that sibutramine increases extracellular noradrenaline in the frontal cortex, with maximum concentrations being reached at least 1 h post-injection and persisting for the duration of the experiment (Wortley *et al*., 1999). However, in our previous study on anaesthetized rats, the relationship between dose of sibutramine and magnitude of response was described by a bell-shaped curve, with a maximum increase in noradrenaline of 278% at 0.5 mg kg^{-1}. In the present investigation, the magnitude of effect of sibutramine paralleled the dose, with a maximum increase of 518% at 10 mg kg^{-1}. One possible hypothesis to explain this shift in the dose-response curve is a change in the function of α_2-adrenoceptors in the presence of anaesthesia, possibly that α_2-adrenoceptors become more sensitive to changes in extracellular noradrenaline concentration under anaesthesia. Thus, in the present study using conscious rats, the dose of sibutramine needed to induce an extracellular noradrenaline concentration sufficient to turn-off locus coeruleus firing was greater than in the previous study using anaesthetized rats. Other explanations for the discrepancy include differences in the dimensions of the synaptic cleft and differences in heteroceptor populations on the noradrenergic neurones innervating these two brain areas.

Sibutramine also increased extracellular noradrenaline in the hypothalamus at 1 and 10 mg kg^{-1}. The time-course for the increase in extracellular noradrenaline induced by these doses are comparable with ED_{50} values for the inhibition of food-intake following a single dose of sibutramine (ED_{50} values: 0.6, 2.0 and 3.7 mg kg^{-1} i.p. at 1, 2 and 4 h post-injection, respectively, in-house data). These findings are both in agreement and at variance with existing literature. Firstly, they are consistent with *in vitro* evidence that the active metabolites of this drug are potent inhibitors of noradrenaline uptake (Cheetham *et al*., 1996). Nevertheless, the finding that sibutramine increases noradrenaline in the region of the PVN is at variance with a long-standing hypothesis that increasing extracellular noradrenaline in this hypothalamic region markedly facilitates feeding behaviour (Matthews *et al*., 1977; Leibowitz, 1978). This effect is thought to be mediated by noradrenergic activation of postsynaptic α_2-adrenoceptors (Goldman *et al*., 1985). However, other studies show that stimulation of α_1-adrenoceptors in the PVN reliably and dose-dependently suppresses feeding behaviour in rats (Wellman, 1992). Therefore, it is probable that the anti-obesity effects induced by sibutramine involve noradrenergic activation of α_1-adrenoceptors in the PVN. Indeed, the hypophagic response to sibutramine in rats is fully reversed by the α_1-adrenoceptor antagonist, prazosin, but is unaltered by the α_2-adrenoceptor antagonist, RX821002 (Jackson *et al*., 1997a).

The magnitude of effect of sibutramine in the hypothalamus of freely-moving rats paralleled dose. However, unlike its action in the cortex, sibutramine induced a more rapid increase in extracellular noradrenaline in the hypothalamus, with a maximum effect occurring 40 min post-injection after a dose of 10 mg kg^{-1}. Following this, dialysate noradrenaline concentrations gradually declined. Different responses from noradrenergic neurones terminating in these two brain areas have been reported before, following chemical and non-chemical stimuli (Zaczek *et al*., 1990; Heal *et al*., 1993; McQuade and Stanford, personal communication). One possible explanation for the contrast observed here is a difference in the pharmacological properties of the noradrenaline transporter site in the cortex and hypothalamus (Jonsson *et al*., 1981; Zaczek *et al*., 1990). Other possibilities could also include differences in the number of noradrenaline transporter sites per noradrenergic neurone and/or differences in heteroceptor populations and their influence on noradrenaline release in the two brain areas.

A further factor which could explain, or contribute to, the different response by cortical and hypothalamic noradrenergic neurones to sibutramine, could involve differences in the function of their α_2-adrenoceptors. The hypothalamus has a greater proportion of presynaptic α_2-adrenoceptors than the cortex (Heal *et al*., 1993); this could imply that noradrenergic neurones in the hypothalamus are subject to greater feedback-inhibition of release by these α_2-adrenoceptors, than are neurones in the cortex. If a greater density of presynaptic α_2-adrenoceptors means a greater control of noradrenaline release, then this could account for the more transient increase in extracellular noradrenaline concentration induced by sibutramine in the hypothalamus compared with the cortex. To explore this possibility, we studied the effects of sibutramine in combination with RX821002. Systemic RX821002 increased resting concentrations of extracellular noradrenaline in both the cortex and hypothalamus by approximately 2 fold. Similarly, local administration of RX821002 into the cortex or hypothalamus approximately doubled basal noradrenaline concentrations. These results are consistent with previous studies in the cortex and hypothalamus using atipamezole (Dalley & Stanford, 1995; Laitinen *et al*., 1995; Gobert *et al*., 1997). Together, the present findings suggest that α_2-adrenoceptors on neurones projecting to both these brain areas are activated under resting conditions and restrict basal noradrenaline release to the same extent. Furthermore, these results confirm that there are α_2-adrenoceptors located in the terminal fields of noradrenergic neurones, most likely on the noradrenergic terminals themselves.

Systemic pretreatment of rats with RX821002 augmented the increase in extracellular noradrenaline concentration induced by sibutramine in both the cortex and hypothalamus. However, there were again differences between the responses of the two brain areas. In the cortex, RX821002 reduced the latency for sibutramine to reach its peak effect. This is consistent with the disinhibition of noradrenaline release by this α_2-adrenoceptor antagonist (Wortley *et al*., 1999). Gobert *et al*. (1997) also found α_2-adrenoceptor blockade to potentiate the effects of another noradrenaline and 5-HT reuptake inhibitor, duloxetine, on extracellular noradrenaline concentrations in the cortex. However, in the present study there was no apparent reduction in the latency for sibutramine to reach its peak effect in the hypothalamus, although it should be borne in mind that it is unlikely a reduction would have been detected, due to the collection of dialysate samples at 20 min intervals.

Because previous reports suggest that RX821002 has moderate affinity for 5-HT_{1A} receptors (Vauquelin *et al*., 1990; Javier-Meana *et al*., 1996), we repeated experiments involving the systemic administration of RX821002 with another highly selective α_2-adrenoceptor antagonist, atipamezole. This drug has negligible affinity for 5-HT_{1A} receptors

(Winter & Rabin, 1992) and it produced effects on extracellular noradrenaline almost identical to RX821002 when combined with sibutramine. Therefore, we are confident that the observed changes in extracellular noradrenaline induced by RX821002 were a result of α_2-adrenoceptor blockade and, subsequently, local perfusion experiments were carried out with RX821002 only.

Local infusion of RX821002 into the terminal fields augmented the increase in extracellular noradrenaline induced by sibutramine, in both the cortex and hypothalamus. These findings agree with a previous report that local infusion of an α_2-adrenoceptor antagonist into the cortex can potentiate the effects of the noradrenaline reuptake inhibitor, desipramine (Dennis *et al*., 1987). However, infusion of RX821002 into the hypothalamus caused a 3 fold increase above the noradrenaline concentration induced by sibutramine alone, compared with a 1.5 fold increase when infused into the cortex. This finding is consistent with evidence that there is a greater density of presynaptic α_2-adrenoceptors in the hypothalamus than in the cortex (Heal *et al*., 1993). Although both populations of α_2-adrenoceptors appear equally effective at restricting basal noradrenaline release, the greater density of α_2-adrenoceptors in the hypothalamus could be more able to control large increases in extracellular noradrenaline, such as that induced by sibutramine. This hypothesis is supported by the finding that RX821002-infusion into the hypothalamus also prolonged the increase in extracellular noradrenaline concentration induced by sibutramine in this brain area. Presynaptic α_2-adrenoceptors in the hypothalamus could control increases in extracellular noradrenaline concentration more effectively than in the cortex by reducing noradrenaline release more quickly. This would account for the different time-courses in extracellular noradrenaline accumulation induced by sibutramine in these two brain areas.

In conclusion, sibutramine induces a gradual and sustained increase in extracellular noradrenaline concentration in the frontal cortex of conscious, freely moving rats. It also increases extracellular noradrenaline in the hypothalamus, although this increase is more rapid and shorter-lived than in the cortex. The increases in noradrenaline induced by sibutramine in both areas are restricted by α_2-adrenoceptors, although terminal α_2-adrenoceptors in the hypothalamus appear to cause a greater restriction to sibutramine's effects on noradrenaline than do terminal α_2-adrenoceptors in the cortex. This could explain the different responses to sibutramine of noradrenergic neurones terminating in these two brain areas.

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