British Journal of Pharmacology (1999) 127, 1860–1866
http://www.stockton-press.co.uk/bjp

Comparison of changes in the extracellular concentration of noradrenaline in rat frontal cortex induced by sibutramine or *d*-amphetamine: modulation by α_2-adrenoceptors

[1]K.E. Wortley, [1]Z.A. Hughes, [2]D.J. Heal & *,[1]S.C. Stanford

[1]Department of Pharmacology, University College London, Gower Street, London WC1E 6BT and [2]Knoll Pharmaceuticals Research and Development, Pennyfoot Street, Nottingham NG1 1GF

1 The effects of sibutramine (0.25–10 mg kg^{-1}, i.p.) on extracellular noradrenaline concentration in the frontal cortex of halothane-anaesthetized rats were compared with those of *d*-amphetamine (1–3 mg kg^{-1}, i.p.) using *in vivo* microdialysis. The role of presynaptic α_2-adrenoceptors in modulating the effects of these drugs on extracellular noradrenaline concentration were also investigated by pretreating rats with the selective α_2-adrenoceptor antagonist, RX821002.

2 Sibutramine induced a gradual and sustained increase in extracellular noradrenaline concentration. The dose-response relationship was described by a bell-shaped curve with a maximum effect at 0.5 mg kg^{-1}. In contrast, *d*-amphetamine induced a rapid increase in extracellular noradrenaline concentration, the magnitude of which paralleled drug dose.

3 Pretreatment with the α_2-adrenoceptor antagonist, RX821002 (dose 3 mg kg^{-1}, i.p.) increased by 5 fold the accumulation of extracellular noradrenaline caused by sibutramine (10 mg kg^{-1}) and reduced the latency of sibutramine to reach its maximum effect from 144–56 min.

4 RX821002-pretreatment increased by only 2.5 fold the increase in extracellular noradrenaline concentration caused by *d*-amphetamine alone (10 mg kg^{-1}) and had no effect on the latency to reach maximum.

5 These findings support evidence that sibutramine acts as a noradrenaline uptake inhibitor *in vivo* and that the effects of this drug are blunted by indirect activation of presynaptic α_2-adrenoceptors. In contrast, the rapid increase in extracellular noradrenaline concentration induced by *d*-amphetamine is consistent with this being mainly due to an increase in Ca^{2+}-independent release of noradrenaline.

Keywords: Sibutramine; noradrenaline: *d*-amphetamine; α_2-adrenoceptors; frontal cortex; *in vivo* microdialysis

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

Introduction

The anti-obesity drug 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 through the enhancement of thermogenesis (Stock, 1997). Evidence from experiments involving selective monoamine reuptake inhibitors and receptor antagonists suggests that sibutramine's ability to increase satiety and thermogenesis is dependent upon the combined inhibition of 5-hydroxytryptamine (5-HT) and noradrenaline reuptake in the central nervous system (Jackson *et al*., 1997a,b; Heal & Cheetham, 1997; Stock, 1997).

In vitro, sibutramine is a weak inhibitor of noradrenaline and 5-HT uptake (Cheetham *et al*., 1993; 1996). However, when administered to either rats or humans, sibutramine is rapidly demethylated into 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) (Luscombe *et al*., 1989; Heal *et al*., 1998a). These active metabolites are potent uptake inhibitors of both noradrenaline and 5-HT *in vitro* (Cheetham *et al*., 1993; 1996) and *in vivo* (Luscombe *et al*., 1989) and predominantly mediate the pharmacological effects of sibutramine *in vivo* (Luscombe *et al*., 1989). *In vitro* evidence also indicates that, in contrast to weight-modifying agents like *d*-fenfluramine and *d*-amphetamine, neither sibutramine nor its active metabolites act as noradrenaline or 5-HT releasing agents (Heal *et al*., 1998a). This hypothesis is supported by evidence from *in vivo* microdialysis experiments showing that, like the selective serotonin reuptake inhibitors, fluoxetine and paroxetine, sibutramine produces a moderate, gradual and sustained increase in extracellular 5-HT, which is subject to autoreceptor feedback control (Gundlah *et al*., 1997; Heal *et al*., 1998b). This contrasts with the massive, rapid and comparatively short-lasting increase in extracellular 5-HT evoked by 5-HT releasing agents e.g. fenfluramine, *d*-fenfluramine and *d*-amphetamine (Gundlah *et al*., 1997; Heal *et al*., 1998b).

Although both *in vitro* and *ex vivo*, sibutramine and its metabolites have been characterized as noradrenaline reuptake inhibitors devoid of releasing actions (Heal *et al*., 1998a), there have been no previous studies of sibutramine's effects on extracellular noradrenaline *in vivo*. The first aim of this investigation was to use the technique of microdialysis to determine whether sibutramine increases the extracellular concentration of noradrenaline in the brain.

The noradrenaline releasing agent *d*-amphetamine (Paton, 1975; Heal *et al*., 1998a) also has established anorectic actions (Sanghvi *et al*., 1975) which have been attributed, in part, to its

*Author for correspondence; E-mail: C.Stanford@ucl.ac.uk

activity on central noradrenergic neurones (Samanin & Garattini, 1993). However, central noradrenergic neurones are also involved in the behavioural activation induced by this drug (Kuczenski & Segal 1992). In fact, there is evidence that it is these psychostimulant properties of *d*-amphetamine, rather than any direct effect on satiety that accounts for the reduction in food intake (Halford *et al*., 1995).

To investigate whether the effects of sibutramine on central noradrenergic transmission could be distinguished from those of *d*-amphetamine, *in vivo* microdialysis was used to compare the changes in the concentration of extracellular noradrenaline induced by these two drugs. However, one limitation of microdialysis is that changes in the concentration of extracellular transmitter can be attributed to either an increase in noradrenaline release, inhibition of its reuptake, or both. Nevertheless, we reasoned that the increase in the concentration of extracellular noradrenaline caused by sibutramine, like that of 5-HT, would be progressive and sustained, as a result of the extracellular accumulation following impulse-evoked release and inhibition of reuptake. In contrast, *d*-amphetamine increases noradrenaline release directly (Paton, 1975; Heal *et al*., 1998a) through a Ca^{2+}-independent mechanism. This involves inhibition of the vesicular transmembrane transporter, which sequesters cytoplasmic amines (Sulzer & Rayport, 1990), and reverse efflux of cytoplasmic noradrenaline on the membrane transporter (Rudnick & Wall, 1992). In view of these different neurochemical actions of sibutramine and *d*-amphetamine, we predicted that the rate of increase in the concentration of extracellular noradrenaline caused by these two compounds would also differ.

Another factor that influences the accumulation of extracellular noradrenaline is activation of somatodendritic and terminal α_2-adrenoceptors on noradrenergic neurones (Gobert *et al*., 1997). When activated by extracellular noradrenaline, these receptors depress the firing rate of noradrenergic neurones (Svensson *et al*., 1975) and release of transmitter from their terminals (Langer, 1977). Therefore, accumulation of extracellular noradrenaline, as a result of the inhibition of its reuptake by sibutramine, should activate these receptors and blunt its impulse-evoked release. In contrast, direct, Ca^{2+}- (impulse)-independent release of noradrenaline by *d*-amphetamine should be less susceptible (if at all) to activation of α_2-adrenoceptors by noradrenaline. Considering this, a further objective of the present study was to compare the effects of systemic administration of the selective α_2-adrenoceptor antagonist, 2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole hydrochloride (RX821002) (Langin *et al*., 1990) on the amplitude and rate of increase in extracellular noradrenaline in the frontal cortex caused by systemic administration of sibutramine or *d*-amphetamine in rats.

Methods

Animals

Male outbred Sprague-Dawley rats (250–320 g) were obtained from the colony at University College London. They 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

Microdialysis probes were constructed with a 5 mm length of semipermeable membrane (i.d. 200 mm, o.d. 300 mm, mol. wt. cut off 20 kD: Filtral 12, Hospal Industrie, France) essentially as described by Sandberg *et al*. (1986). A tracheal cannula was implanted under halothane-anaesthesia and used to administer freely inspired halothane (1–1.5%) mixed in 95% oxygen and 5% CO_2 at 500 ml min^{-1}. The core body temperature was maintained at 37°C throughout the experiment using a homeothermic heating blanket and rectal probe (Harvard Instruments). Rats were placed in a Kopf stereotaxic frame and the skull exposed to reveal bregma. 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_2 PO_4 0.27, glucose 7.2) was lowered vertically into the frontal cortex (AP +3.5, L −1.5, DV −5.0, Paxinos & Watson, 1986). At the end of the experiment the brain was removed and placed in 10% formaldehyde before probe location was verified histologically using a low-power light microscope.

Microdialysis

The microdialysis 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. Experiments began with the collection of four baseline ('basal') samples; this was followed by administration of a test drug, after which samples were collected for a further 4 h. In experiments involving pretreatment with the selective α_2-adrenoceptor antagonist, RX821002, rats were divided into four groups. The first group received 1 mg kg^{-1} (i.p.) RX821002; 2 h later they received a second dose of 3 mg kg^{-1} (i.p.) RX821002 and samples collected for another 2 h. The second, third and fourth groups of rats received a dose of 3 mg kg^{-1} (i.p.) RX821002 and, 1 h later, were given a further injection of sibutramine, *d*-amphetamine or vehicle; samples were then collected for a further 3 h.

HPLC analysis of dialysate

The noradrenaline content of the dialysate samples was analysed using reverse-phase high pressure liquid chromatography coupled to electrochemical detection. Solutes were separated at room temperature using a Hypersil ODS 5 mm 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 SpectraPhysics 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

All drugs were dissolved in 0.9% saline and administered by intraperitoneal (i.p.) injection in a volume of 2 ml kg^{-1}. The following drugs were used: *d*-amphetamine sulphate (Sigma, U.K.), 2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1H-imidazole 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 with 'time' as the 'within-subjects' factor. If a main effect of time was exposed over the full time course of the experiment, the data were divided into bins of four consecutive samples and bins 2–4 compared with that containing the basal noradrenaline samples (bin 1). 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 again 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

Sibutramine

The mean noradrenaline content of the basal samples was 24.8 ± 0.8 fmol 20 min^{-1}. There was no change in dialysate noradrenaline concentration in rats injected with saline vehicle (Figure 1) but, following administration of sibutramine at 0.25, 0.5 or 1 mg kg^{-1} (Figure 1A) or 1, 3 or 10 mg kg^{-1} (Figure 1B), there was a gradual and sustained increase in the noradrenaline concentration in the dialysis samples.

Sibutramine (0.5, 1 or 3 mg kg^{-1}) increased noradrenaline concentration within 100 min post-injection, (0.5 mg kg^{-1}: $F=7.86$; d.f. 1,7; $P=0.03$; 1 mg kg^{-1}: $F=8.79$; d.f. 1,6; $P=0.03$; 3 mg kg^{-1}: $F=6.30$; d.f 1,8; $P=0.04$). However, sibutramine (0.25 mg kg^{-1}) did not increase dialysate noradrenaline concentration until 100–160 min post-injection ($F=12.29$; d.f. 1,7; $P=0.01$). At no point during the experiment did sibutramine (10 mg kg^{-1}) affect dialysate noradrenaline concentration when compared with the basal samples.

The relationship between the magnitude of the increase and dose of sibutramine followed a bell-shaped curve. The largest increase was induced by a dose of 0.5 mg kg^{-1} (maximum of 278%, *c.f.* basals) and occurred at 240 min post-injection ($F=18.31$; d.f. 1,6; $P=0.01$). A plateau, representing an approximately 2.5 fold increase in noradrenaline (*c.f.* basals), was attained within 2 h of injection. Slightly smaller increases were found after administration of 1 or 3 mg kg^{-1} sibutramine (1 mg kg^{-1}: maximum of 245% at 100 min post-injection: 3 mg kg^{-1}: maximum of 266% at 200 min post-injection).

d-Amphetamine

All three doses of *d*-amphetamine increased the concentration of extracellular noradrenaline within 80 min of injection (1 mg kg^{-1}: $F=8.1$; d.f. 1,6; $P=0.03$; 3 mg kg^{-1}: $F=14.15$; d.f. 1,6; $P=0.01$; 10 mg kg^{-1}: $F=247.3$; d.f. 1,4; $P=0.01$) and the magnitude of the increase paralleled drug dose (Figure 1C). Doses of 1 or 3 mg kg^{-1} produced maximum increases of 281% at 60 min post-injection and 381% at 80 min post-

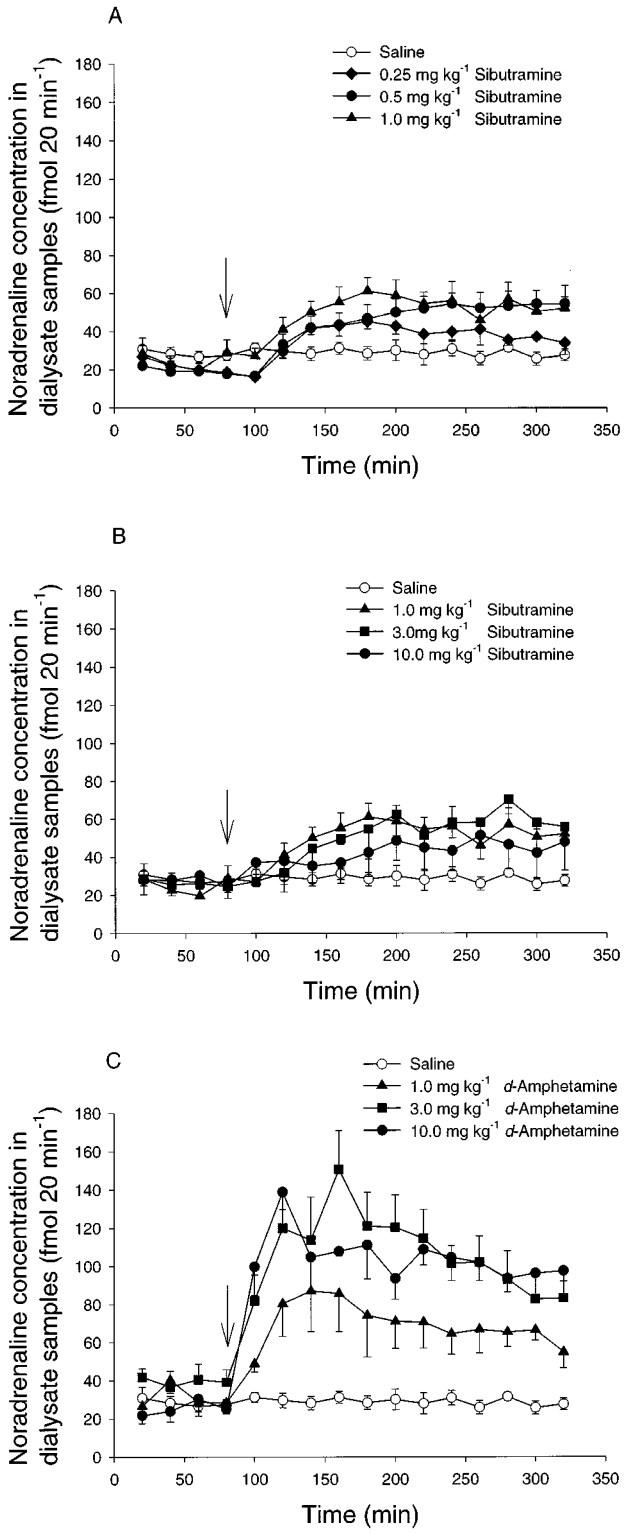

Figure 1 The effects of sibutramine (A: 0.25, 0.5 and 1 mg kg^{-1} or B: 1, 3 and 10 mg kg^{-1}) or *d*-amphetamine (C: 1, 3 and 10 mg kg^{-1}) on the concentration of noradrenaline in samples of cortical dialysate. Arrow represents point of drug administration. Data are expressed as mean$\pm$s.e.mean dialysate noradrenaline concentration (fmol 20 min^{-1}; $n=4-5$).

injection, respectively. The largest increase was induced by a dose of 10 mg kg^{-1} (549% at 40 min post-injection: F = 247.34; d.f. 1,4; $P < 0.01$ *c.f.* basal samples).

Comparison of the effects of sibutramine and d-*amphetamine*

There was no overall difference in the effects of 1 or 3 mg kg^{-1} sibutramine and *d*-amphetamine but *d*-amphetamine (10 mg kg^{-1}) caused a greater increase in dialysate noradrenaline concentration than did sibutramine at all times post-injection (0–80 min: F = 24.71; d.f. 1,5; $P < 0.01$, 100–160 min: F = 12.19; d.f. 1,6; $P = 0.01$, 180–240 min: F = 8.11; d.f. 1,7; $P = 0.03$). There was also a marked difference in the time course of the responses to a 10 mg kg^{-1} dose of these drugs (drug × time interaction: F = 14.59; d.f. 13,65; $P < 0.01$) reflecting the more rapid increase caused by *d*-amphetamine.

Effects of RX821002-pretreatment

The mean noradrenaline content of these basal samples was 22.6 ± 1.2 fmol 20 min^{-1}. Neither RX821002 alone (1 mg kg^{-1} followed by 3 mg kg^{-1}), nor when followed by a saline injection, 1 h later, affected extracellular noradrenaline (Figure 2). However, both sibutramine (10 mg kg^{-1}) and *d*-amphetamine (10 mg kg^{-1}) increased dialysate noradrenaline concentration when administered to RX821002-pretreated rats (Figure 2). Moreover, the increase caused by both these compounds was greater than with either drug alone (sibutramine: ~5 fold increase, F = 12.85; d.f. 1,30; $P < 0.01$; Figure 3A; *d*-amphetamine: ~2.5 fold increase, F = 15.85; d.f. 1,24; $P < 0.01$; Figure 3B).

RX821002 also reduced the latency of sibutramine (10 mg kg^{-1}) to reach its maximum effect from 144 ± 14 min to only 56 ± 4 min (F = 33.80; d.f. 1,8; $P < 0.01$). In contrast, RX821002 had no significant effect on latency to reach mean maximum noradrenaline concentration after treatment with *d*-amphetamine (RX821002 pretreated: 50 ± 17 min non-pretreated: 40 ± 0 min). Finally, a drug × time interaction (F = 2.7; d.f. 3,65; $P < 0.01$) again suggests that, even in the presence of RX821002, the increase in extracellular noradrenaline concentration induced by *d*-amphetamine was more rapid than that caused by sibutramine.

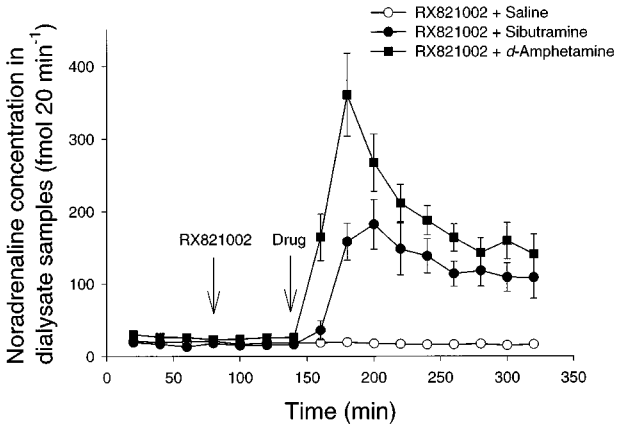

Figure 2 The effects of sibutramine or *d*-amphetamine (10 mg kg^{-1}) on the concentration of noradrenaline in samples of cortical dialysate from rats treated 1 h previously with RX821002 (3 mg kg^{-1}). Arrows represent point of drug administration. Data are expressed as mean ± s.e.mean dialysate noradrenaline concentration (fmol 20 min^{-1}; $n = 5$).

Discussion

The anti-obesity drug, sibutramine causes weight loss through a dual action affecting food intake (Halford *et al*., 1995; Jackson *et al*., 1997a,b) and resting energy expenditure (Stock, 1997). It has been demonstrated that both these effects result from the simultaneous inhibition of neuronal reuptake of 5-HT and noradrenaline in the central nervous system (Jackson *et al*., 1997a,b; Heal & Cheetham, 1997). In contrast, the decrease in food intake induced by the noradrenaline-releasing agent, *d*-amphetamine, has been shown to be secondary to the behavioural activation caused by this psychostimulant (Halford *et al*., 1995). Although the increase in behavioural activation induced by *d*-amphetamine will inevitably increase energy expenditure, this mechanism is quite different from the thermogenic action of sibutramine which increases resting energy expenditure by selective activation of sympathetic drive to brown adipose tissue (Stock, 1997).

In vitro, sibutramine's active metabolites are potent inhibitors of noradrenaline reuptake (Cheetham *et al*., 1996) but both the parent compound and its metabolites are devoid of noradrenaline-releasing activity (Heal *et al*., 1998a). However, nothing is known about the effects of sibutramine on extracellular noradrenaline *in vivo*. Questions that arise are

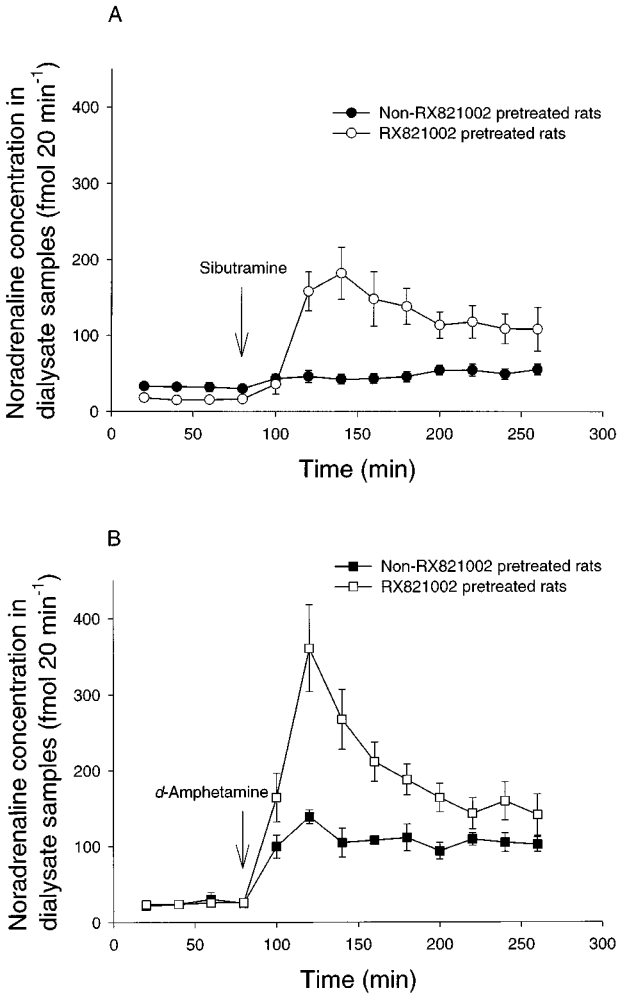

Figure 3 Comparison of the effects of (A) sibutramine (10 mg kg^{-1}) or (B) *d*-amphetamine (10 mg kg^{-1}) on the concentration of noradrenaline in samples of cortical dialysate from non-pretreated rats and rats treated 1 h previously with RX821002 (3 mg kg^{-1}). Arrow represents point of drug administration. Data are expressed as mean ± s.e.mean dialysate noradrenaline concentration (fmol 20 min^{-1}; $n = 5$).

whether its reuptake blocking activity increases extracellular noradrenaline and whether this action can be distinguished from the effect of *d*-amphetamine?

The present results show that sibutramine causes a gradual and sustained increase in the concentration of extracellular noradrenaline in rat frontal cortex. *d*-Amphetamine also increased extracellular noradrenaline concentration. However, at the highest dose tested (10 mg kg^{-1}), this increase was both more rapid and of greater magnitude than that caused by the same dose of sibutramine. The magnitude and time-course of effects of these two drugs on extracellular noradrenaline concentration resemble their effects on extracellular 5-HT in the hypothalamus (Gundlah *et al*., 1997). Also, as in the present study of noradrenaline, the profile of the increase in extracellular 5-HT concentration evoked by 5-HT-reuptake inhibitors differs markedly from that induced by a 5-HT-releasing agent (Gundlah *et al*., 1997). On the basis of these findings, Gundlah *et al*. (1997) defined the specific criteria which distinguish between the effects of 5-HT-reuptake inhibitors and 5-HT-releasing agents when using microdialysis. According to these criteria: (1) Systemic administration of a releasing agent induces a greater increase in extracellular 5-HT concentration than that induced by reuptake inhibitors. (2) Attenuation of the firing of 5-HT neurones, through activation of presynaptic autoreceptors, diminishes the effects of reuptake inhibitors on extracellular 5-HT concentration but does not affect the actions of releasing agents because these depend on impulse-independent transmitter release.

Applying these criteria to the findings from the present study suggests that *d*-amphetamine produces a greater and more rapid increase in extracellular noradrenaline concentration than does sibutramine because the former drug is a releasing agent whereas the latter is a reuptake inhibitor, only. Thus, the rapid increase caused by *d*-amphetamine is mainly due to impulse-independent release of noradrenaline (Sulzer & Rayport, 1990; Florin *et al*., 1994) whereas the more gradual increase caused by sibutramine reflects the progressive accumulation of noradrenaline after its spontaneous, impulse-evoked release.

An additional caveat is that several reports suggest that *d*-amphetamine actually decreases the firing rate of noradrenergic neurones in the locus coeruleus (Graham & Aghajanian, 1971; Ryan *et al*., 1985; Holdefer & Jensen, 1987). It has further been suggested that such a depression of neuronal firing reduces activation of presynaptic α_2-adrenoceptors which, in turn, disinhibits release of noradrenaline from the terminals (Ryan *et al*., 1985; Curet *et al*., 1992). Therefore, the possibility that impulse-dependent release contributes to the increase in extracellular noradrenaline induced by *d*-amphetamine, cannot be ruled out. Notwithstanding this complication, the findings reported so far lead to the prediction that activation of presynaptic α_2-adrenoceptors, which modulate release of noradrenaline, would blunt the response to sibutramine whereas the effects of *d*-amphetamine would be less susceptible, if at all.

When given alone, the α_2-adrenoceptor antagonist, RX821002, did not modify basal dialysate noradrenaline concentration. This suggests that there is little tonic activation of these receptors. This finding is at variance with reports that systemic administration of RX821002 does increase the concentration of extracellular noradrenaline in rat brain (Meana *et al*., 1997; Nutt *et al*., 1997). However, whereas Meana *et al*. (1997) report a somewhat larger increase in extracellular noradrenaline after treatment with RX821002 (Meana *et al*., 1997), the results of this study cannot be compared with those reported here because the noradrenaline reuptake inhibitor, desipramine, was included in the aCSF. This, in itself, would indirectly increase the tonic activation of α_2-adrenoceptors.

Nutt *et al*. (1997) have also reported a 2 fold increase in extracellular noradrenaline after administration of RX821002. One explanation for our apparently anomalous finding is that, unlike the present study, that of Nutt *et al*. (1997) was carried out under chloralose anaesthesia. Noradrenaline efflux is affected in different ways by different anaesthetic agents and chloralose appears to decrease the concentration of extracellular noradrenaline in the hypothalamus, at least (Shimokawa *et al*., 1998). Consistent with this, the concentration of noradrenaline in the basal samples of the present study is approximately 2 fold higher than that reported in Nutt *et al*. (1997). This could possibly be because chloralose, like many anaesthetic agents, augments the function of α_2-adrenoceptors. Despite evidence that halothane anaesthesia also modifies α_2-adrenoceptor function (Kenny *et al*., 1989; Larach *et al*., 1987; Wikberg *et al*., 1987), this tentative explanation is supported by results from a separate study in which we have investigated the effects of systemic RX821002 on extracellular noradrenaline concentration in the frontal cortex of conscious, freely-moving rats (Wortley *et al*. unpublished). Under these conditions, RX821002 did cause a 2 fold increase in extracellular noradrenaline concentration. Finally, there is extensive evidence that α_2-adrenoceptor antagonists, such as yohimbine or idazoxan (L'Heureux *et al*., 1986; Thomas & Holman, 1991; Mason *et al*., 1997), increase the extracellular concentration of noradrenaline. However, neither of these agents is selective for α_2-adrenoceptors; in fact, RX821002 was used in this study specifically because of its comparatively low affinity for other binding sites, including imidazoline receptors (Langin *et al*., 1990).

Despite its lack of intrinsic activity, RX821002 augmented the increase in extracellular noradrenaline concentration caused by sibutramine. This finding suggests that activation of somatodendritic and/or terminal α_2-adrenoceptors by extracellular noradrenaline, which accumulates after treatment with sibutramine, attenuates impulse-derived transmitter release. In keeping with these findings, the increase in cortical extracellular noradrenaline concentration caused by the established noradrenaline reuptake inhibitor, desipramine, is similarly enhanced by idazoxan (Dennis *et al*., 1987).

RX821002 also augmented the effect of *d*-amphetamine (10 mg kg^{-1}) on extracellular noradrenaline. This suggests that impulse-evoked release of noradrenaline does contribute to the actions of this drug to some extent. This finding supports electrophysiological evidence that low doses of *d*-amphetamine can increase impulse-dependent release of noradrenaline (Ryan *et al*., 1985; Curet *et al*., 1992). However, this inference conflicts with evidence that α_2-adrenoceptor agonists have little effect on the increase in noradrenaline induced by *d*-amphetamine in the hippocampus of conscious rats (Florin *et al*., 1994). This could be because activation of α_2-adrenoceptors after treatment with *d*-amphetamine is already maximal and that co-administration of an α_2-adrenoceptor agonist has no further impact on release rate.

Pretreatment of rats with RX821002 not only augmented the amplitude of the increase in extracellular noradrenaline caused by sibutramine, but also reduced the latency to reach its maximum. This is consistent with disinhibition of impulse-derived noradrenaline release by this α_2-adrenoceptor antagonist. There was no apparent reduction in the latency to reach maximum after treatment with *d*-amphetamine from the 40 min seen in the RX821002-free state. However, it is unlikely that any such reduction would have been detected because the

present protocol for microdialysis dictated sampling at 20 min intervals.

In conclusion, the findings of this study support the view that sibutramine is a noradrenaline reuptake inhibitor *in vivo* and that the rate and magnitude of this drug's effects on extracellular noradrenaline are attenuated by indirect activation of α_2-adrenoceptors. In contrast, *d*-amphetamine causes a much more rapid increase in extracellular noradrenaline; the majority, but not all, of this increase is attributed to impulse-independent release of noradrenaline.

References

CHEETHAM, S.C., VIGGERS, J.A., SLATER, N.A., HEAL, D.J. & BUCKETT, W.R. (1993). [^{3}H]Paroxetine binding in rat frontal cortex strongly correlates with [^{3}H]5-HT uptake: Effect of administration of various antidepressant treatments. *Neuropharmacology,* **32,** 737–743.

CHEETHAM, S.C., VIGGERS, J.A., BUTLER, S.A., PROW, M.R. & HEAL, D.J. (1996). [^{3}H]Nisoxetine-A radioligand for noradrenaline reuptake sites: Correlation with inhibition of [^{3}H]noradrenaline uptake and effect of DSP-4 lesioning and antidepressant treatments. *Neuropharmacology,* **35,** 63–70.

CURET, O., DE MONTIGNY, C. & BLIER, P. (1992). Effects of desipramine and amphetamine on noradrenergic neurotransmission: electrophysiological studies in the rat brain. *Eur. J. Pharmacol.,* **221,** 59–70.

DENNIS, T., L'HEUREUX, R., CARTER, C. & SCATTON, B. (1987). Presynaptic d_2-adrenoceptors play a major role in the effects of idazoxan on cortical noradrenaline (as measured by in vivo dialysis) in the rat. *J. Pharmacol. Exp. Ther.,* **241,** 642–649.

FLORIN, S.M., KUCZENSKI, R. & SEGAL, D.S. (1994). Regional extracellular norepinephrine responses to amphetamine and cocaine and effects of clonidine pretreatment. *Brain. Res.,* **654,** 53–62.

GOBERT, A., RIVET, J.M., CISTARELLI, C., MELON, C. & MILLAN, M.J. (1997). d_2-Adrenergic receptor blockade markedly potentiates duloxetine- and fluoxetine-induced increases in noradrenaline, dopamine, and serotonin levels in the frontal cortex of freely-moving rats. *J. Neurochem.,* **69,** 2616–2619.

GRAHAM, A.W. & AGHAJANIAN, G.K. (1971). Effects of amphetamine on single cell activity in a catecholamine nucleus, the locus coeruleus. *Nature,* **234,** 100–102.

GUNDLAH, C., MARTIN, K.F., HEAL, D.J. & AUERBACH, S.B. (1997). *In vivo* criteria to differentiate monoamine uptake inhibitors from releasing drugs: Sibutramine is a reuptake inhibitor. *J. Pharmacol. Exp. Ther.,* **283,** 581–591.

HALFORD, J.C.G., HEAL, D.J. & BLUNDELL, J.E. (1995). Effects in the rat of sibutramine on food intake and the behavioural satiety sequence. *Br. J. Pharmacol.,* **114,** 387P.

HEAL, D.J. & CHEETHAM, S.C. (1997). The pharmacology of sibutramine, the first serotonin and noradrenaline reuptake inhibitor to be developed for the treatment of obesity. *La lettre du pharmacologue,* **11,** (suppl 10), 3–8.

HEAL, D.J., ASPLEY, S., PROW, M.R., JACKSON, H.C., MARTIN, K.F. & CHEETHAM, S.C. (1998a). Sibutramine: a novel anti-obesity drug. A review of the pharmacological evidence to differentiate it from *d*-amphetamine and *d*-fenfluramine. *Int. J. Obesity,* **22,** (Suppl 1), S18–S28.

HEAL, D.J., CHEETHAM, S.C., PROW, M.R., MARTIN, K.F. & BUCKETT, W.R. (1998b). A comparison of the effects on central 5-HT function of sibutramine hydrochloride and other weight-modifying agents. *Br. J. Pharamacol.,* **125,** 301–308.

HOLDEFER, R.N. & JENSEN, R.A. (1987). The effects of peripheral D-amphetamine, 4-OH amphetamine, and epinephrine on maintained discharge in the locus coeruleus with reference to the modulation of learning and memory by these substances. *Brain Res.,* **417,** 108–117.

JACKSON, H.C., BEARHAM, M.C., HUTCHINS, L.J., MAZURKIEWICZ, S.E., NEEDHAM, A.M. & HEAL, D.J. (1997a). Investigation of the mechanisms underlying the hypophagic effects of the 5-HT and noradrenaline reuptake inhibitor, sibutramine, in the rat. *Br. J. Pharmacol.,* **121,** 1613–1618.

JACKSON, H.C., NEEDHAM, A.M., HUTCHINS, L.J., MAZURKIEWICZ, S.E. & HEAL, D.J. (1997b). Comparison of the effects of sibutramine and other monoamine reuptake inhibitors on food intake in the rat. *Br. J. Pharmacol.,* **121,** 1758–1762.

KENNY, D., PELC, L.R., BROOKS, H.L., KAMPINE, J.P., SCHMELING, W.T. & WARLTIER, D.C. (1989). Alterations of d_1- and d_2-adrenoceptor-mediated pressor responses by halothane and isoflurane anaesthesia. *Anaesthesiology,* **71,** 224–234.

KUCZENSKI, R. & SEGAL, D.S. (1992). Regional norepinephrine response to amphetamine using dialysis: Comparison with caudate dopamine. *Synapse,* **11,** 164–169.

LANGER, S.Z. (1977). Sixth Gaddum memorial lecture, National Institute for Medical Research, Mill Hill, Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmacol.,* **60,** 481–497.

LANGIN, D., PARIS, H. & LAFONTAN, M. (1990). Binding of [3H]idazoxan and of its methoxy derivative [3H]RX821002 in human fat cells: [3H]idazoxan but not [3H]RX821002 labels additional non-alpha2-adrenergic binding sites. *Mol. Pharmacol.,* **37,** 876–885.

LARACH, D.R., SCHULER, H.G., DERR, J.A., LARACH, M.G., HENSLEY, F.A. & ZELIS, R. (1987). Halothane selectively attenuates d_2-adrenoceptor mediated vasoconstriction in vivo and in vitro. *Anaesthesiology,* **66,** 781–791.

L'HEUREUX, R.L., DENNIS, T., CURET, O. & SCATTON, B. (1986). Measurement of endogenous noradrenaline release in the rat cerebral cortex in vivo by transcortical dialysis: Effects of drugs affecting noradrenergic transmission. *J. Neurochem.,* **46,** 1794–1801.

LUSCOMBE, G.P., HOPCROFT, R.H., THOMAS, P.C. & BUCKETT, W.R. (1989). The contribution of metabolites to the rapid and potent down-regulation of rat cortical β-adrenoceptors by the putative antidepressant sibutramine hydrochloride. *Neuropharmacology,* **28,** 129–134.

MASON, K., HEAL, D.J. & STANFORD, S.C. (1997). The anxiogenic agents, yohimbine and FG 7142, disrupt the noradrenergic response to novelty. *Pharm. Biochem Behav.,* **60,** 321–327.

MEANA, J.J., HERRERA-MARSCHITZ, M., GOINY, M. & SILVEIRA, R. (1997). Modulation of catecholamine release by alpha2-adrenoceptors and I1-imidazoline receptors in rat brain. *Brain. Res.,* **744,** 216–226.

NUTT, D.J., LALIES, M.D., LIONE, L.A. & HUDSON, A.L. (1997). Noradrenergic mechanisms in the prefrontal cortex. *J. Psychopharmacol.,* **11,** 163–168.

PATON, D.M. (1975). Structure-activity relations for the acceleration of efflux of noradrenaline from adrenergic nerves in rabbit atria by sympathomimetic amines. *Can. J. Physiol. Pharmacol.,* **53,** 822–829.

PAXINOS, G. & WATSON, C. (1986). The rat brain in stereotaxic coordinates. Academic Press, Australia.

RUDNICK, G. & WALL, S.C. (1992). *p*-Chloroamphetamine induces serotonin release through serotonin transporters. *Biochemistry,* **31,** 6710–6718.

RYAN, L.J., TEPPER, J.M., YOUNG, S.J. & GROVES, P.M. (1985). Amphetamine's effects on terminal excitability of noradrenergic locus coeruleus neurones are impulse-dependent at low but not high doses. *Brain Res.,* **341,** 155–163.

SAMANIN, R. & GARATTINI, S. (1993). Neurochemical mechanism of action of anorectic drugs, *Pharmacol. Toxicol.,* **73,** 63–68.

SANDBERG, M., BUTCHER, S.P. & HAGBERG, H. (1986). Extracellular overflow of neuroactive amino acids during severe insulin-induced hypoglycemia: in vivo dialysis of the rat hippocampus. *J. Neurochem.,* **47,** 178–184.

SANGHVI, I.S., SINGER, G., FRIEDMAN, E. & GERSHON, S. (1975). Anorexigenic effects of d-amphetamine and l-DOPA in the rat. *Pharmacol. Biochem. Behav.,* **3,** 81–86.

SHIMOKAWA, A., JIN, Q.H., ISHIZUKA, Y., KUNITAKA, T., TAKASAKI, M. & KANNAN, H. (1998). Effects of anesthetics on norepinephrine release in the hypothalamus paraventricular nucleus region of awake rats. *Neurosci. Lett.,* **244,** 21–24.

STOCK, M.J. (1997). Sibutramine: a review of the pharmacology of a novel antiobesity agent. *Int. J. Obes.,* **21** (Suppl. 1), S25–S29.

SVENSSON, T.H., BUNNEY, B.S. & AGHAJANIAN, G.K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain. Res.,* **92,** 291–306.

SULZER, D. & RAYPORT, S. (1990). Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: a mechanism of action. *Neuron,* **5,** 797–808.

THOMAS, D.N. & HOLMAN, R.B. (1991). A microdialysis study of the regulation of endogenous noradrenaline release in the rat hippocampus. *J. Neurochem.,* **56,** 1741–1746.

WIKBERG, J.E.S., HEDE, A.R. & POST, C. (1987). Effects of halothane and other chlorinated hydrocarbons on alpha2-adrenoceptors in the mouse cortex. *Pharmacol. Toxicol.,* **61,** 271–277.

(Received 11 March, 1999
Revised 13 May, 1999
Accepted 17 May, 1999)