ELSEVIER

European Journal of Pharmacology 445 (2002) 69–81

ejp

www.elsevier.com/locate/ejphar

Effects on serotonin in rat hypothalamus of D-fenfluramine, aminorex, phentermine and fluoxetine

Rui Tao [a], Anne Fray [b], Sue Aspley [b], Richard Brammer [b], David Heal [b], Sidney Auerbach [a,*]

[a] *Nelson Laboratories, Department of Cell Biology and Neuroscience, Rutgers University, 604 Allison Road, Piscataway, NJ 08854-8082, USA*
[b] *Knoll Limited, Research and Development, Nottingham, UK*

Received 21 February 2002; received in revised form 25 April 2002; accepted 30 April 2002

Abstract

Hypothalamic 5-HT (serotonin) regulates food intake, energy expenditure and bodyweight. Using in vivo microdialysis, we determined the effects of various anorectic drugs on hypothalamic extracellular 5-HT levels during the dark phase when rats predominantly feed. Phentermine and aminorex, which were originally considered to be catecholaminergic drugs, markedly increased 5-HT efflux in rat hypothalamus. Their actions were less profound than D-fenfluramine, but considerably greater than that of the selective 5-HT reuptake inhibitor, fluoxetine. This suggests that enhanced hypothalamic 5-HT function could be involved in their anorectic actions. Pharmacological characterization revealed that D-fenfluramine, aminorex and probably also phentermine potentiate synaptic 5-HT function predominantly by release, whereas fluoxetine acts exclusively by reuptake inhibition. The results also revealed that the combined actions of phentermine and D-fenfluramine on hypothalamic extracellular 5-HT levels were additive, but not synergistic. In contrast, there was a significant negative cooperative effect on extraneuronal 5-HT of combining phentermine with fluoxetine.

Keywords: 5-Hydroxytryptamine (5-HT, serotonin); Microdialysis; Anti-obesity agent; Serotonin uptake inhibitor; Amphetamine; Fenfluramine

1. Introduction

It is well known that the hypothalamus is the central regulator of food intake and energy expenditure (for reviews, see Leibowitz and Alexander, 1998; Schwartz et al., 2000; Williams et al., 2000). Furthermore, serotonin (5-hydroxytryptamine, 5-HT) plays a key role in modulating food intake in animals and man (for reviews, see Blundell and Halford, 1998; Leibowitz and Alexander, 1998). This fact is emphasised by the known hypophagic actions of drugs which enhance central 5-HT function, including fenfluramine and its enantiomer, D-fenfluramine (Borsini et al., 1985; Rowland and Carlton, 1988; Samanin et al., 1989) and the 5-HT selective reuptake inhibitors, fluoxetine (Clifton et al., 1989; Caccia et al., 1992; Lee and Clifton, 1992) and sertraline (Lucki et al., 1988; Grignaschi and Samanin, 1993). Fenfluramine and D-fenfluramine are effective weight-loss agents in man and were used extensively to treat obesity until an association with primary pulmonary hypertension and cardiac valvulopathy (Abenhaim et al., 1996; Connolly et al., 1997) led to their withdrawal in 1997. High doses of the reuptake inhibitor fluoxetine have also been shown to produce clinically significant weight-loss in obese subjects for up to 6 months, but its development as an anti-obesity agent was discontinued because its efficacy was not maintained at 1 year (Goldstein et al., 1994). An important development in the search for more effective anti-obesity therapies has been the potential synergistic interaction between 5-HT and other monoamine neurotransmitters in the control of energy regulation and bodyweight; the use of the phentermine/fenfluramine (phen/fen) combination (Weintraub, 1992) and the development of the 5-HT and noradrenaline reuptake inhibitor, sibutramine, (McNeely and Goa, 1998) are examples of these approaches.

Several recent in vivo microdialysis studies have been performed to investigate the effects of phentermine, the phen/fen combination and aminorex (another anti-obesity drug that was withdrawn for causing primary pulmonary hypertension) on central 5-HT function. However, these experiments have not been performed in the hypothalamus, but in other brain regions, i.e. hippocampus (Zheng et al., 1997), striatum (Balcioglu and Wurtman, 1998a,b) and nucleus accumbens (Rothman et al., 1999; Baumann et al., 2000). Whilst the data provide useful information, these

* Corresponding author. Tel.: +1-732-445-3441; fax: +1-732-445-5870.
E-mail address: auerbach@biology.rutgers.edu (S. Auerbach).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01751-X

brain regions are not key to energy regulation and the reported findings do not necessarily apply to the hypothalamus. In support of this contention, it has been recently shown that the actions of both monoamine reuptake inhibitors and releasing agents on noradrenaline efflux differ markedly between the hypothalamus and frontal cortex (Wortley et al., 1999). Another important factor is that rats are predominantly nocturnal feeders (Boulos and Terman, 1979) and hypothalamic 5-HT efflux and its autoreceptor control show a marked circadian variance (Martin and Marsden, 1985; Martin and Redfern, 1997; Sayer et al., 1999; Garabette et al., 2000).

In the light of the above information, we have now conducted an experimental study to determine the effects of D-fenfluramine, phentermine, aminorex and fluoxetine on the extraneuronal levels of 5-HT in the hypothalamus of conscious, freely moving rats. To define the pharmacological mode of action of these drugs, i.e. 5-HT reuptake or release, we have used the experimental approaches that we previously described (Gundlah et al., 1997). We have also studied the phen/fen and more recently proposed phentermine/fluoxetine (Prozac®) (phen/Pro) combinations to elucidate whether they have either an additive or synergistic effect on extraneuronal 5-HT. Finally, all of the experiments have been conducted under conditions of reversed-phase lighting in order to define these parameters under conditions most relevant to the feeding pattern of rats.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats purchased from Harlan Sprague Dawley (Indianapolis, IN) were individually housed with food and water available ad libitum. The animals were kept on a reversed light/dark cycle (lights off from 9:30 a.m. to 21:30 p.m.) and all experiments were performed during the dark phase. All animal use procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Rutgers University Institutional Review Board. Rats weighing 300–350 g were anesthetized with a combination of xylazine (4 mg/kg, intraperitoneally, i.p.) and ketamine (80 mg/kg, i.p.), and guide cannulae (21-gauge stainless steel tubing) were implanted as previously described in detail (Auerbach et al., 1989). After surgery, the guide cannulae were plugged with stylets and the rats were allowed a recovery period of at least 1 week.

2.2. Microdialysis procedures

Microdialysis was performed with a concentric design probe constructed from 26-gauge stainless steel tubing and glass silica. The dialysis tubing was hollow nitrocellulose fiber (0.25 mm o.d., 13,000 MW cut-off; Spectrum Medical Industries, Los Angeles, CA). The length of the steel shaft was adjusted to place the tip of a 3.5-mm-long segment of dialysis tubing slightly above the base of the hypothalamus (flat skull position: 6.2 mm anterior and 0.9 mm lateral relative to intra-aural zero and 9.2 mm below dura) (Paxinos and Watson, 1986).

The evening before an experiment, rats were briefly anesthetized with ether, and dialysis probes were inserted and secured with dental cement. Rats were then placed in the test chamber, and attached to a counter-weighted cable and swivel that allowed animals to move freely and have access to food and water. Before collecting samples, dialysis probes were perfused overnight with a modified, buffered Ringer solution containing 140 mM NaCl, 3.0 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.27 mM NaH_2PO_4, 1.2 mM Na_2HPO_4, pH 7.4. This Ringer solution (artificial cerebrospinal fluid) was pumped at a rate of 1.0 μl/min. Sample collection began at the beginning of the lights-off period under dim red light conditions.

Samples were collected every 30 min and analyzed by high-performance liquid chromatography with electrochemical detection. Separation of 5-HT was achieved on a 10 cm × 3.2 mm column with ODS III 3 μm packing (BAS, West Lafayette, IN). The mobile phase composition was 0.12 M NaOH, 0.18 mM EDTA, 0.15 M monochloroacetic acid, 1.0 mM sodium octane sulfonic acid and 56 ml/l acetonitrile, pH 3.4, and was pumped at a rate of 0.90 ml/min. Monoamines were measured using a dual potentiostat electrochemical detector (EG&G PARC, Oak Ridge, TN) and dual glassy carbon electrodes in the parallel configuration. Applied potentials, relative to an Ag/AgCl electrode, were set at approximately maximal and half-maximal for oxidation of 5-HT. These values were checked frequently and were usually about 590 and 530 mV. The detection limit for 5-HT was approximately 0.3 pg/sample based on a signal-to-noise ratio of 3:1.

2.3. Experimental design and data analysis

Mean baseline 5-HT levels were calculated as the average of the four successive samples before drug administration and reported in the figure legends as pg/sample, uncorrected for probe recovery. Also, the data were normalized and presented in figures as mean (± S.E.M.) percent change from the averaged baseline measurements. For each figure, an *F*-test was applied first to determine whether or not the treatment groups were significantly different from each other. Further, statistical analysis of individual time-points was carried out by analysis of covariance (ANCOVA) with baseline as the covariate and treatment as the factor. Data were log transformed to ensure normality of distributions as required for parametric statistics. Comparisons between drug treatment and control groups were made using Williams' test when there was a dose–response; otherwise, the multiple *t*-test was used. The effect of administering phentermine alone compared to phentermine combined with D-

fenfluramine (Fig. 5B,D) or fluoxetine (Fig. 6B,D) was evaluated using mean values for samples taken between 0 and 3 h after drug injection. To determine if phentermine combined with D-fenfluramine or fluoxetine had multiplicative effects on 5-HT, interactions were defined by the formula (all terms are mean percentages of baseline 5-HT levels):

$$100\% \times \frac{\text{Combination/Phentermine alone}}{\text{D} - \text{Fenfluramine or fluoxetine alone/Saline}}$$

According to this formula, 100% represents a "null effect" of the combination of phentermine with D-fenfluramine or fluoxetine. Using analysis of covariance to test the interaction contrast, numbers significantly greater than 100% indicate more than multiplicative effects, and numbers significantly less than 100% indicate less than multiplicative effects.

2.4. Drugs and reagents

All chemicals were "reagent grade" or better. Fluoxetine, aminorex, D-fenfluramine and (±)-8-hydroxy-2-dipropylaminotetralin (8-OH-DPAT) were purchased from RBI (Natick, MA). Phentermine was obtained from Sigma (St. Louis, MO). Drugs were administered to rats after 5-HT levels in four successive samples were stable (less than ± 10% fluctuation of baseline). For systemic administration, all drugs except 8-OH-DPAT were dissolved in physiological saline (0.9% NaCl) and were injected via the intraperitoneal route. 8-OH-DPAT was dissolved in distilled water and was injected subcutaneously. For reverse dialysis infusion into hypothalamus, drugs were dissolved in artificial cerebrospinal fluid.

3. Results

3.1. Effect of systemic administration of fluoxetine, D-fenfluramine, aminorex and phentermine on extracellular 5-HT levels

In the first set of experiments, we compared the relative potency, efficacy and time-course of 1–10 mg/kg doses of

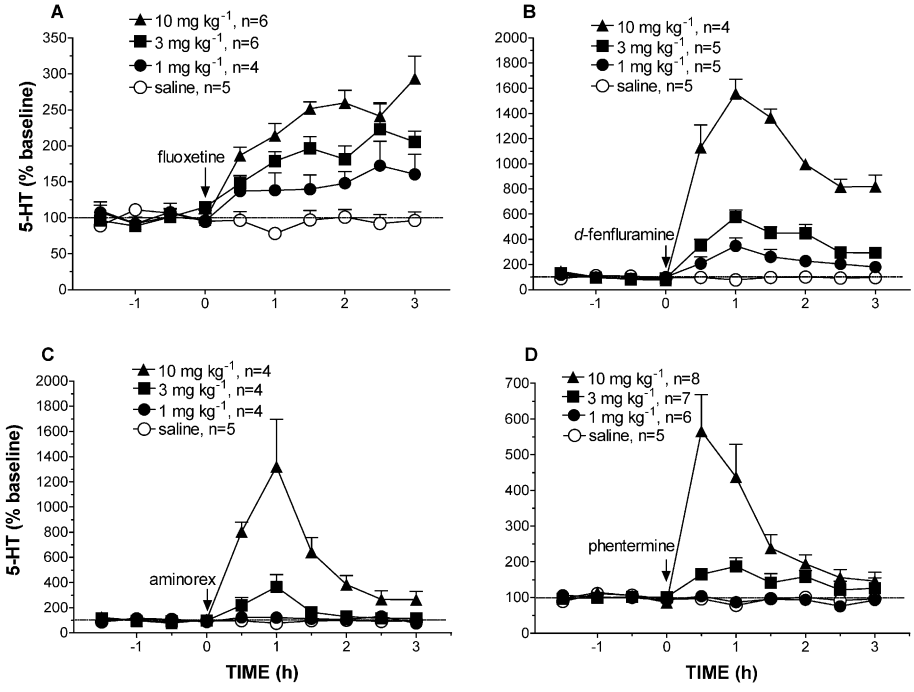

Fig. 1. Effect of fluoxetine, D-fenfluramine, aminorex and phentermine on extracellular 5-HT in the hypothalamus. Means (± S.E.M.) are expressed as a percentage of the average of four baseline samples. Note the use of different *y*-axis ranges within this figure. Drugs or vehicle were administered at time 0 (arrow). All treatment groups (12 drug groups and a common saline vehicle group) were found to be significantly different from each other; $F(12,54) = 26.9$, $P < 0.001$. (A) The mean baseline levels of 5-HT for the three fluoxetine treatment groups were not significantly different and the combined value was 1.4 ± 0.3 pg/sample. Compared to saline controls, 5-HT was significantly increased in response to fluoxetine (1 mg/kg, $P = 0.02$; 3 mg/kg, $P < 0.001$; 10 mg/kg, $P < 0.001$). (B) The mean baseline levels of 5-HT for the three D-fenfluramine treatment groups were not significantly different and the combined value was 1.1 ± 0.1 pg/sample. Compared to saline controls, 5-HT was significantly increased in response to D-fenfluramine (1 mg/kg, $P < 0.001$; 3 mg/kg, $P < 0.001$; 10 mg/kg, $P < 0.001$). (C) The mean baseline levels of 5-HT for the three aminorex treatment groups were not significantly different and the combined value was 1.5 ± 0.2 pg/sample. Compared to saline controls, 5-HT was not significantly increased in response to aminorex at 1 mg/kg ($P = 0.31$), but was significantly increased in response to the higher doses ($P < 0.001$ at both 3 and 10 mg/kg). (D) The mean baseline levels of 5-HT for the three phentermine treatment groups were not significantly different and the combined value was 1.1 ± 0.1 pg/sample. Compared to saline controls, 5-HT was not significantly increased in response to phentermine at 1 and 3 mg/kg ($P = 0.41$ and 0.13, respectively), but was significantly increased in response to the highest dose (10 mg/kg, $P < 0.001$).

phentermine, aminorex, D-fenfluramine and fluoxetine to increase extracellular levels of 5-HT in the hypothalamus of freely moving rats when measured during the early phase of the dark period.

Fluoxetine produced an increase in extracellular 5-HT in the hypothalamus after systemic administration (Fig. 1A). This effect was dose-dependent, with injections of 1, 3 and 10 mg/kg producing maximum increases of ~ 50%, 100% and 200% above baseline, respectively. Levels slowly increased to a maximum between 2 and 3 h after injecting fluoxetine. Systemic administration of D-fenfluramine, phentermine and aminorex also produced dose-dependent increases in extracellular 5-HT in the hypothalamus (Fig. 1B–D). In contrast to fluoxetine, 5-HT levels had peaked by 1 h after drug injection and then declined towards baseline levels. At doses of 1, 3 and 10 mg/kg, D-fenfluramine increased 5-HT to maximum levels above baseline of ~ 250%, 500% and 1500%, respectively (Fig. 1B). Neither phentermine nor aminorex elicited a significant increase in 5-HT when administered at a dose of 1 mg/kg. However, 5-HT was increased to a maximum of ~ 300% and 1200% above baseline after injection of aminorex at doses of 3 and 10 mg/kg, respectively (Fig. 1C). Similar to D-fenfluramine, the maximum increase was observed at 1 h after injection and then gradually returned to baseline. In response to phentermine at doses of 3 and 10 mg/kg, 5-HT was increased to ~ 100% and 500% above baseline, respectively (Fig. 1D). However, the maximum elevation was seen at the first sample (0.5 h) immediately after injection.

3.2. Effect of 8-OH-DPAT on the increase in extracellular 5-HT produced by fluoxetine, D-fenfluramine, phentermine and aminorex

In order to define whether the drug-induced increases in extracellular 5-HT were dependent or independent of neuronal firing, we used the 5-HT_{1A} autoreceptor agonist, 8-OH-DPAT, to inhibit 5-HT neuronal activity.

Administration of 8-OH-DPAT (0.1 mg/kg, s.c.) at the same time as fluoxetine (10 mg/kg, i.p.) significantly attenuated the initial increase in extracellular 5-HT (Fig. 2A). This effect of 8-OH-DPAT lasted for ~ 1.5 h, with

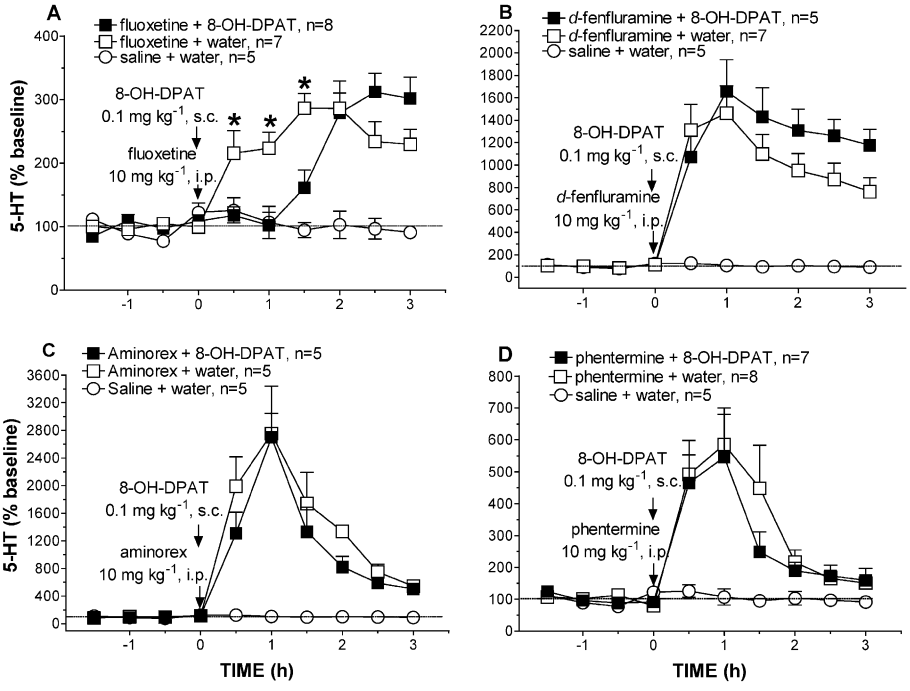

Fig. 2. Effect of 8-OH-DPAT (0.1 mg/kg, s.c.) on fluoxetine-, D-fenfluramine-, aminorex- or phentermine-induced increases in hypothalamic 5-HT. Means (± S.E.M.) are expressed as a percentage of the average of four baseline samples. Note the use of different *y*-axis ranges within this figure. All drugs were administered at time 0 (arrow). For the critical time period of 0–90 min of 8-OH-DPAT effect on 5-HT, across all treatment groups (8 drug groups and a common saline vehicle group), there was a significant difference; $F(8,47)=29.2$, $P<0.001$. (A) The mean baseline levels of 5-HT for the two fluoxetine treatment groups were not significantly different and the combined value was 1.1 ± 0.1 pg/sample. Administration of 8-OH-DPAT significantly attenuated the increase in response to fluoxetine (10 mg/kg, i.p.) for samples collected from time 0 through 1.5 h after injection; * $P=0.005$. (B) The mean baseline levels of 5-HT for the two D-fenfluramine treatment groups were not significantly different and the combined value was 1.3 ± 0.2 pg/sample. Administration of 8-OH-DPAT had no significant effect on the D-fenfluramine (10 mg/kg, i.p.)-induced increase in 5-HT; $P=0.92$. (C) The mean baseline levels of 5-HT for the two aminorex treatment groups were not significantly different and the combined value was 0.9 ± 0.1 pg/sample. Administration of 8-OH-DPAT had no significant effect on the aminorex (10 mg/kg, i.p.)-induced increase in 5-HT; $P=0.34$. (D) The mean baseline levels of 5-HT for the two phentermine treatment groups were not significantly different and the combined value was 1.4 ± 0.2 pg/sample. Administration of 8-OH-DPAT had no significant effect on the phentermine (10 mg/kg, i.p.)-induced increase in 5-HT; $P=0.22$.

Table 1
In vitro 5-HT transporter affinities and release profiles in rat brain tissues

Drug	[^{3}H]5-HT inhibition, K_i (nM)	[^{3}H]5-HT release (% above basal overflow)	
		1000 nM	10,000 nM
Fluoxetine	11[a]	–	–
D-Fenfluramine	280[b]	64[b]	282[b]
Phentermine	11,200[b]	–	–
Aminorex	1380[b]	51[b]	116[b]

Table shows K_i values for rat cortical synaptosomes and [^{3}H]5-HT release from preloaded rat cortical slices measured by superfusion.
[a] Data previously reported in Heal et al. (1998).
[b] Data previously reported in Lancashire et al. (1998).

5-HT increasing to ~200% above baseline at 2 h after drug injection. From 2 h to the end of the experiment, there was no significant difference between the rats treated with 8-OH-DPAT and the control group injected with fluoxetine alone. In contrast, 8-OH-DPAT had no significant influence on the magnitude or time-course of the effect of D-fenfluramine (10 mg/kg, i.p.) on 5-HT (Fig. 2B). Similarly, 8-OH-DPAT did not alter the effect of aminorex (10 mg/kg, i.p.; Fig. 2C) or phentermine (10 mg/kg, i.p.; Fig. 2D).

3.3. Effect of local infusion followed by systemic injection of fluoxetine, D-fenfluramine, phentermine and aminorex

The dependence of the actions of these drugs on 5-HT neuronal firing was further defined by determining whether increased 5-HT efflux evoked by reverse dialysis of the compound into the 5-HT terminal fields of hypothalamus was modified by intraperitoneal injection of the compound. The design of this experiment is based on evidence that the local effect of a reuptake inhibiting drug in terminal sites is dependent on neuronal firing and thus attenuated by its peripheral injection (Hjorth and Auerbach, 1994; Rutter et al., 1995) because increased extraneuronal concentrations of 5-HT at the somatodendritic level inhibit neuronal firing (Gartside et al., 1995). The concentration of phentermine, aminorex and D-fenfluramine used for local infusion was 10-fold higher than fluoxetine. This was necessary because of the much lower affinities of these compounds for the 5-HT transporter as

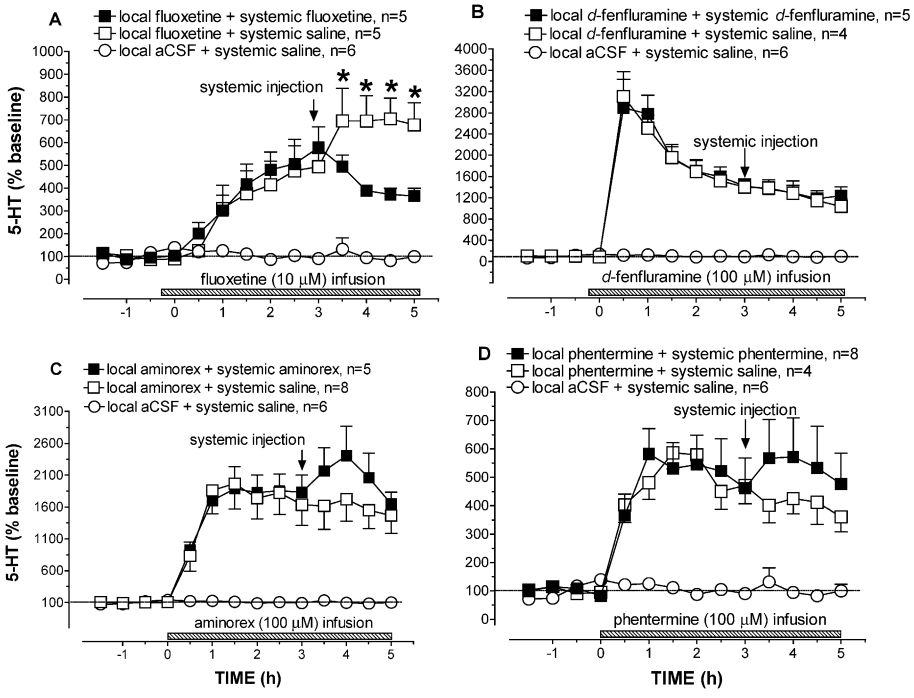

Fig. 3. Effect of systemic administration of fluoxetine, D-fenfluramine, aminorex or phentermine on the increase in extracellular 5-HT produced by local infusion of these drugs into the hypothalamus. Means (± S.E.M.) are expressed as a percentage of the average of four baseline samples. The mean baseline levels of 5-HT for the fluoxetine, D-fenfluramine, aminorex and phentermine experiments were 1.1 ± 0.1, 1.3 ± 0.1, 1.1 ± 0.1 and 1.1 ± 0.2 pg/sample, respectively. Note the use of different *y*-axis ranges within this figure. Beginning at time 0, fluoxetine (A), D-fenfluramine (B), aminorex (C) or phentermine (D) were infused by reverse dialysis into the hypothalamus (horizontal bars). For the period of local drug infusion, across all treatment groups (4 drug groups and a common saline vehicle group), there was a significant difference; $F(4,45) = 78.3$, $P < 0.001$. Between 0 and 3 h, local infusion of these drugs produced significant increases in extracellular 5-HT: fluoxetine (10 μM; $P < 0.001$); D-fenfluramine (100 μM; $P < 0.001$); aminorex (100 μM; $P < 0.001$); phentermine (100 μM; $P < 0.001$). At $t = 3$ h (arrows), either the corresponding drug (10 mg/kg) or saline was injected intraperitoneally. For the period after systemic drug injection, across all treatment groups (4 drug groups and a common saline vehicle group), there was a significant difference; $F(4,42) = 8.72$, $P < 0.001$. Compared to the saline control group, fluoxetine produced a significant decrease in 5-HT (*$P < 0.001$). There was no significant effect of systemic D-fenfluramine ($P = 0.89$), aminorex ($P = 0.17$) or phentermine ($P = 0.31$).

shown in Table 1 and a key provision of this experimental protocol that the 5-HT transporter is already maximally affected by local infusion of the drug at the time of systemic administration. Thus, systemic administration should have no further direct effect on 5-HT nerve terminals in the hypothalamus but may have an indirect influence on efflux in the hypothalamus via changes in extracellular 5-HT in the area of somatodendritic autoreceptors.

As shown in Fig. 3A, reverse dialysis infusion of fluoxetine (10 μM dissolved in artificial cerebrospinal fluid) into the hypothalamus increased extracellular 5-HT by ~ 500% above baseline levels and subsequent intraperitoneal injection of fluoxetine (10 mg/kg) significantly decreased this to ~ 50% of 5-HT levels in the control group (10 μM fluoxetine infusion followed by systemic saline injection). In contrast, systemic administration during local infusion of the other anti-obesity drugs did not produce decreases in extracellular 5-HT. Reverse dialysis infusion of D-fenfluramine (100 μM) into the hypothalamus produced an increase to ~ 3000% above baseline 5-HT (Fig. 3B). Extracellular 5-HT was maximal in the first sample during infusion with levels falling rapidly to ~ 1500% above baseline at 3 h. For the remainder of the experiment, 5-HT levels were relatively stable and there was no significant difference between rats injected at 3 h with D-fenfluramine (10 mg/kg) compared to the controls injected with saline (Fig. 3B). Infusion of aminorex (100 μM) into the hypothalamus produced an increase in 5-HT to ~ 1800% above baseline levels. Subsequent peripheral injection of aminorex (10 mg/kg) had no significant effect as compared to the controls injected with saline (Fig. 3C). Infusion of phentermine (100 μM) into the hypothalamus produced a relatively stable increase in 5-HT to ~ 500% above baseline levels, and subsequent peripheral injection of phentermine (10 mg/kg) had no significant effect (Fig. 3D).

3.4. Effect of fluoxetine pretreatment on D-fenfluramine-, phentermine- or aminorex-induced 5-HT release

To determine whether phentermine, aminorex and D-fenfluramine evoke 5-HT efflux secondary to transport into the terminal via the 5-HT reuptake transporter, we examined the effect of using fluoxetine to block the carrier.

Fluoxetine pretreatment attenuated the increase in 5-HT induced by D-fenfluramine (10 mg/kg; Fig. 4A). Thus, extracellular 5-HT was increased only ~ 300% above the post-fluoxetine baseline. This is in contrast to the ~ 1500% increase in 5-HT after systemic injection of D-fenfluramine (10 mg/kg) alone. Similarly, aminorex produced only an ~ 400% increase in 5-HT above the post-fluoxetine baseline, in contrast to the ~ 2500% increase in response to aminorex alone (Fig. 4B). The effect of phentermine (10 mg/kg) on 5-HT was completely blocked by fluoxetine pretreatment (Fig. 4C).

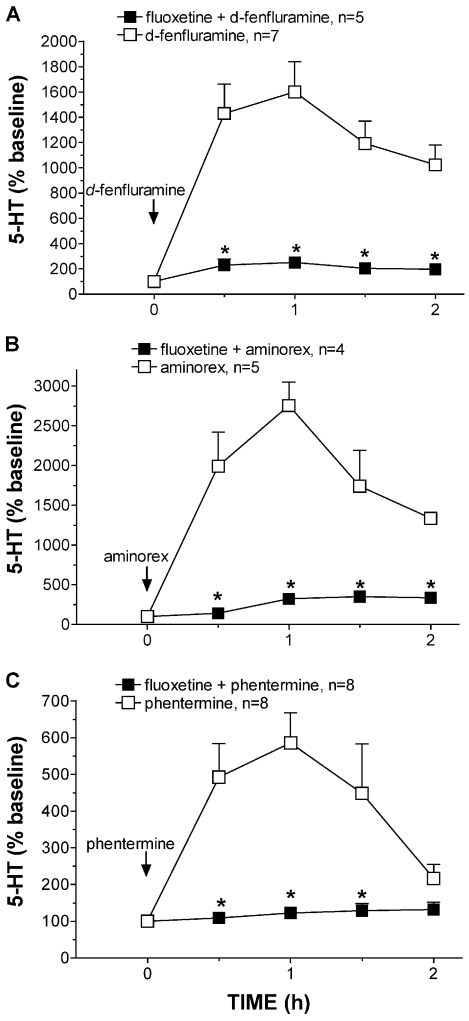

Fig. 4. Effect of pretreatment with fluoxetine on the increase in 5-HT produced by D-fenfluramine, aminorex or phentermine. Fluoxetine (10 mg/kg, i.p.) was injected followed 2 h later by D-fenfluramine (10 mg/kg, i.p.), aminorex (10 mg/kg, i.p.) or phentermine (10 mg/kg, i.p.). Means (± S.E.M.) are expressed as the percentage increase over baseline levels at 2 h after fluoxetine injection. Note the use of different *y*-axis ranges within this figure. Across all treatment groups, there was a significant difference; $F(5,31)=28.1$, $P<0.001$. (A) At the time of D-fenfluramine injection, 2 h after fluoxetine pretreatment, the mean baseline level of 5-HT was 1.7 ± 0.2 pg/sample. For the fluoxetine pretreatment group, D-fenfluramine produced a significantly smaller increase in 5-HT ($P<0.001$). (B) At the time of aminorex injection, 2 h after fluoxetine pretreatment, the mean baseline level of 5-HT was 3.5 ± 0.6 pg/sample. For the fluoxetine pretreatment group, aminorex produced a significantly smaller increase in 5-HT ($P<0.001$). (C) At the time of phentermine injection, 2 h after fluoxetine pretreatment, the mean baseline level of 5-HT was 3.1 ± 0.6 pg/sample. For the fluoxetine pretreatment group, phentermine produced a significantly smaller increase in 5-HT ($P<0.001$).

3.5. Effect of combined administration of phentermine with D-fenfluramine or fluoxetine

The last set of experiments examined the effects of combined simultaneous administration of phentermine with

D-fenfluramine or fluoxetine on extracellular levels of 5-HT in the hypothalamus.

When D-fenfluramine (1 mg/kg) was combined with an equal dose of phentermine (1 mg/kg), the maximal increase in 5-HT was ~ 350% above baseline (Fig. 5A). D-Fenfluramine (1 mg/kg) combined with a separate saline injection evoked a maximal increase to ~ 200% above baseline levels. Phentermine (1 mg/kg) combined with a separate saline injection (1 ml/kg) had no significant effect on 5-HT. To determine the nature of the effects of the combination of D-fenfluramine and phentermine on extracellular 5-HT levels, the overall changes in 5-HT between 0 and 3 h after drug administration were expressed as mean percentages of baseline and an interaction term was calculated as described in Materials and methods. As shown in Fig. 5B, the effect of combined phentermine and D-fenfluramine injection (phen + fen) on extracellular 5-HT levels was significantly greater than the effect of either phentermine alone or D-fenfluramine alone. In combination, the actions of these two drugs on extraneuronal 5-HT tended strongly towards posi-

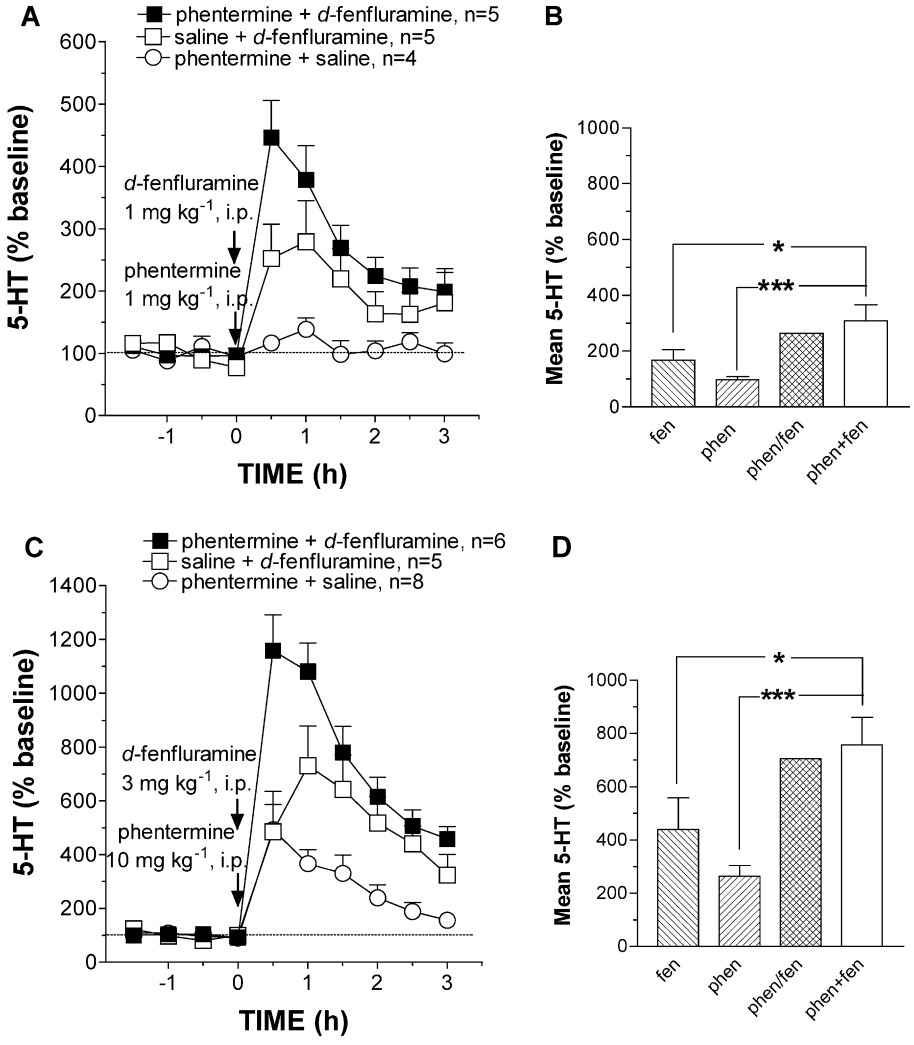

Fig. 5. Effect of combined systemic administration of phentermine with D-fenfluramine on hypothalamic 5-HT. Means (± S.E.M.) are expressed as a percentage of the average of four baseline samples. Note the use of different *y*-axis ranges within this figure. At time 0, phentermine or saline was injected with D-fenfluramine. Across all treatment groups in this figure and in Fig. 6 (including the phentermine at 1 and 10 mg/kg + saline groups shared by this figure and Fig. 6, and the saline vehicle group, data not shown), there was a significant difference; $F(10,49) = 19.3$, $P < 0.001$. (A) The mean baseline levels of 5-HT for the three treatment groups were not significantly different and the combined value was 0.8 ± 0.1 pg/sample. At low doses, combined administration of phentermine (1 mg/kg, i.p.) with D-fenfluramine (1 mg/kg, i.p.) had a greater effect on extracellular 5-HT as compared with phentermine alone ($P < 0.001$) and D-fenfluramine alone ($P < 0.01$). (B) The bar graph shows the mean values from 0 to 3 h after drug injection. The cross-hatched bar (phen/fen) shows the sum of the effects of phentermine (phen; left-hatched bar) and D-fenfluramine (fen; right-hatched bar). * $P = 0.01$ and *** $P < 0.001$ vs. the combined injection group (phen + fen; solid bar), multiple *t*-test. (C) The mean baseline levels of 5-HT for the three treatment groups were not significantly different and the combined value was 1.1 ± 0.1 pg/sample. At high doses, combined administration of phentermine (10 mg/kg, i.p.) with D-fenfluramine (3 mg/kg, i.p.) had a greater effect on extracellular 5-HT compared with injections of phentermine alone ($P < 0.001$) and D-fenfluramine alone ($P < 0.01$). (D) The bar graph shows the mean values from 0 to 3 h after drug injection. The cross-hatched bar (phen/fen) shows the sum of the effects of phentermine (phen; left-hatched bar) and D-fenfluramine (fen; right-hatched bar). * $P = 0.01$ and *** $P < 0.001$ vs. the combined injection group (phen + fen; solid bar), multiple *t*-test.

tive multiplicativity, although this effect did not quite reach statistical significance (interaction value = 184.5 ± 58.4%; $P = 0.06$).

Since the interaction value for the effect of combined injection of phentermine and D-fenfluramine at low doses was not statistically significant, we repeated this experiment using higher doses. When injected separately, phentermine (10 mg/kg) and D-fenfluramine (3 mg/kg) produced increases to a maximum of ~ 400% and ~ 600%, respectively, above baseline (Fig. 5C). In response to combined administration at these doses, 5-HT was increased to a maximum of ~ 1000% above baseline. The overall changes in 5-HT between 0 and 3 h after drug administration were also expressed as mean percentages of baseline, and as shown in Fig. 5D, combined injection of phentermine and D-fenfluramine had simple additive effects on 5-HT. Thus, the increase in response to combined injection (phen + fen) at these higher doses is similar to the sum of the effects of separate injections of phentermine and D-fenfluramine (phen/fen), indicating an additive effect only, with no evidence of synergy (65.3 ± 18.0%; $P = 0.13$).

Further experiments examined the effects of co-administration of phentermine and fluoxetine administration on extracellular 5-HT levels. At a low dose (1 mg/kg; Fig. 6A),

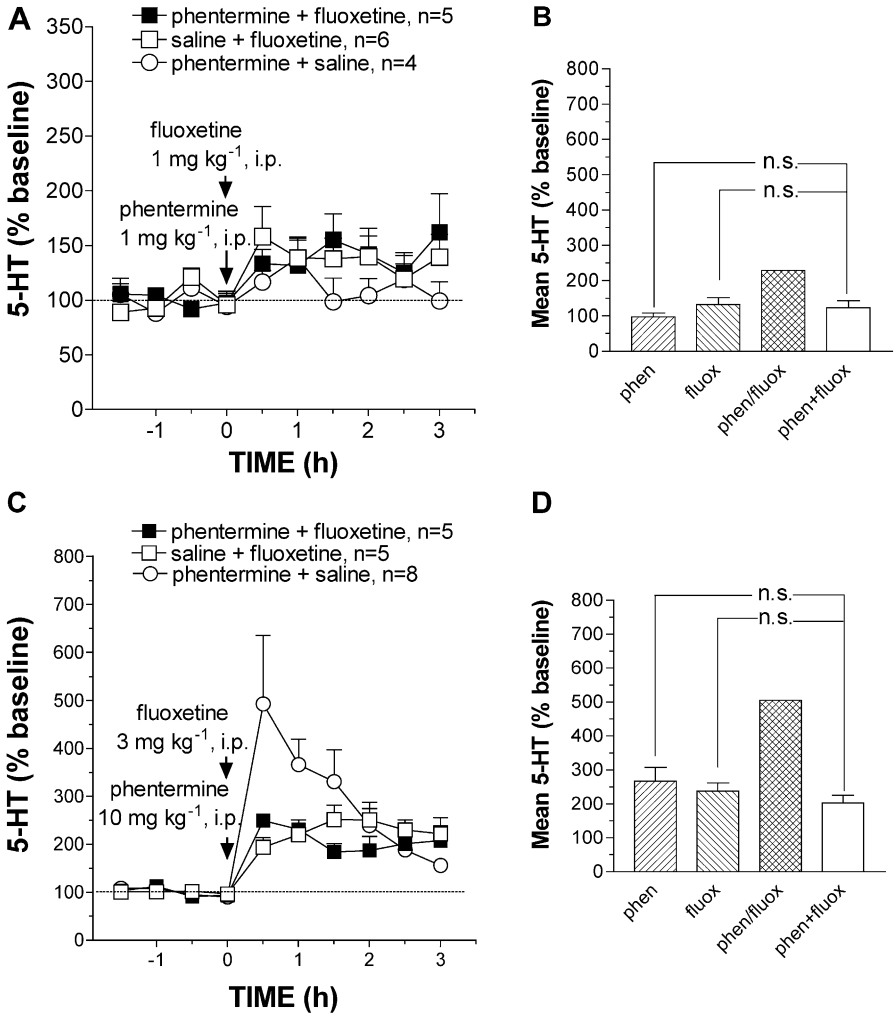

Fig. 6. Effect of combined systemic administration of phentermine with fluoxetine on hypothalamic 5-HT. Means (± S.E.M.) are expressed as a percentage of the average of four baseline samples. Note the use of different *y*-axis ranges within this. At time 0, phentermine or saline was injected with fluoxetine. (A) The mean baseline levels of 5-HT for the three treatment groups were not significantly different and the combined value was 1.0 ± 0.1 pg/sample. At the low dose, administration of phentermine (1 mg/kg, i.p.) combined with fluoxetine (1 mg/kg, i.p.), phentermine alone or fluoxetine alone had no significant effect on 5-HT. (B) The bar graph shows mean values between 0 and 3 h after drug injection. The cross-hatched bar (phen/fluox) shows the sum of the effects of phentermine (phen; left-hatched bar) and fluoxetine (fluox; right-hatched bar). There were no significant differences (n.s.) of the combination compared with the effect of separate injections of fluoxetine and phentermine. (C) The mean baseline levels of 5-HT for the three treatment groups were not significantly different and the combined value was 1.3 ± 0.1 pg/sample. At the high dose, combined injection of phentermine (10 mg/kg, i.p.) and fluoxetine (3 mg/kg, i.p.) had a similar effect on 5-HT compared with phentermine alone. (D) The bar graph shows mean values between 0 and 3 h. The cross-hatched bar (phen/fluox) shows the sum of the effects of phentermine (phen; left-hatched bar) and fluoxetine (fluox; right-hatched bar). There were no significant differences (n.s.) in comparing the effect of combined injection with that of separate injections of fluoxetine and phentermine.

neither phentermine nor fluoxetine alone or in combination produced significant increases in 5-HT in the hypothalamus ($P > 0.05$). For the overall mean data shown in Fig. 6B, the increase in 5-HT in response to combined administration (phen + fluox) was less than the sum of the effects of phentermine and fluoxetine alone (phen/fluox). However, as indicated by the interaction value (101.5 ± 29.6%; $P = 0.96$), there was no evidence that these two compounds had a multiplicative effect at this dose.

Since the increases in 5-HT were small at low doses of phentermine and fluoxetine (Fig. 6A), we tested higher doses of phentermine (10 mg/kg) and fluoxetine (3 mg/kg) administered separately and in combination. As shown in Fig. 6C, in response to phentermine alone, 5-HT increased to a maximal level of ~ 400% above baseline. Fluoxetine either alone or injected together with phentermine produced similar increases to ~ 150% above baseline 5-HT. To determine if there were interactions between the higher doses of phentermine and fluoxetine, the overall changes in 5-HT between 0 and 3 h after drug administration were expressed as mean percentages of baseline. As shown in Fig. 6D, the value for the overall mean of the combined injection appeared much smaller than the sum of the effects of separate injections of phentermine and D-fenfluramine. Moreover, as indicated by an interaction value significantly less than 100% (32.2 ± 8.0%; $P < 0.001$), combined administration had a less than multiplicative effect on 5-HT.

4. Discussion

The aims of the study were threefold. The first aim was to define the relative effects of four drugs that have been tested or registered for the treatment of obesity, phentermine, aminorex, D-fenfluramine and fluoxetine, on the extraneuronal levels of 5-HT in rat hypothalamus. These experiments were conducted during the dark phase of the light/dark cycle because rats are predominantly nocturnal feeders. The second aim was to define the mechanism by which each drug enhances extraneuronal 5-HT, i.e. reuptake inhibition or release. The third aim was to evaluate the impact of combining phentermine with D-fenfluramine (phen/fen) or with fluoxetine (phen/Pro) on extraneuronal levels of 5-HT in the hypothalamus.

It has long been known that the hypothalamus controls food intake and energy expenditure (see reviews of Schwartz et al., 2000; Williams et al., 2000), and furthermore, that 5-HT is a monoamine neurotransmitter which plays a key role in maintaining the energy balance, particularly with respect to regulating food intake (see reviews of Blundell and Halford, 1998; Leibowitz and Alexander, 1998). Drugs that enhance central 5-HT function, e.g. D-fenfluramine and fluoxetine, have been shown to reduce the food consumption of rodents (Rowland and Carlton, 1988; Samanin et al., 1989; Clifton et al., 1989; Caccia et al., 1992; Lee and Clifton, 1992). Whilst the fenfluramines, i.e. DL-fenfluramine and D-fenfluramine, could deliver clinically meaningful weight-loss in patients, attempts were made to improve efficacy by combining drugs with what at the time were perceived as different pharmacological modes of action (Atkinson et al., 1995). The simple approach of combining a serotonergic drug, i.e. fenfluramine, with a catecholaminergic drug, i.e. phentermine, resulted in impressive weight-loss efficacy (Weintraub, 1992), and this finding triggered the widespread use of this combination in the 1990s. However, questions concerning the association of prolonged use of the fenfluramines with primary pulmonary hypertension (Abenhaim et al., 1996), potential neurotoxicity with these compounds (Molliver and Molliver, 1990; McCann et al., 1994; Cheetham et al., 2000) that may be exacerbated by combined administration with phentermine (Lew et al., 1997; McCann et al., 1998), and finally, cardiac valvulopathy (Connolly et al., 1997) led to the worldwide withdrawal of fenfluramine and D-fenfluramine. In the aftermath of this situation, it was acknowledged that our understanding of the pharmacological actions of these 'older' anorectics, particularly phentermine, was poor and it prompted a number of research groups to reinvestigate these drugs, and in addition, to examine other anorectics, e.g. aminorex, that had previously been withdrawn for causing primary pulmonary hypertension. These studies have included a focus on the monoamine oxidase inhibitory properties of phentermine (Ulus et al., 2000; Kilpatrick et al., 2001) and also its ability to potentiate the output of 5-HT from nerve terminals (Shoaib et al., 1997; Zheng et al., 1997; Balcioglu and Wurtman, 1998a,b; Baumann et al., 2000; Rothman et al., 2001).

However, as stated in the Introduction, these experimental investigations are not directly relevant to characterising the actions of these drugs on central 5-HT function in relation to energy regulation. Thus, in the present study, we have performed microdialysis experiments in the hypothalamus and during the dark phase of the light/dark cycle because there are circadian variations in rats feeding pattern (Boulos and Terman, 1979) and basal efflux and autoreceptor control of 5-HT in this brain region (Martin and Marsden, 1985; Martin and Redfern, 1997; Sayer et al., 1999; Garabette et al., 2000).

Under these conditions in rats, we observed pronounced differences between the time course and magnitude of the effect of fluoxetine compared to D-fenfluramine, aminorex and phentermine. The selective 5-HT reuptake inhibitor, fluoxetine, which reduces food consumption in rats (Clifton et al., 1989; Caccia et al., 1992; Lee and Clifton, 1992), produced relatively small, but sustained, increases in extraneuronal 5-HT in the rat hypothalamus. This observation is consistent with our previous findings in this brain region (Gundlah et al., 1997), and those of researchers who have studied other forebrain areas (Dailey et al., 1992; Perry and Fuller, 1992; Malagié et al., 1995) that even high doses of fluoxetine (10–45 mg/kg) will not increase extracellular 5-

HT above ~ 300% of baseline levels. In contrast, D-fenfluramine evoked a marked dose-dependent increase in extraneuronal 5-HT that peaked rapidly to a maximum of ~ 1400% above basal and then slowly declined. A similar pattern of effect on extraneuronal levels of 5-HT in the hypothalamus was previously observed with the racemate, DL-fenfluramine (Gundlah et al., 1997). In the present study, we found that phentermine and aminorex similarly evoke substantial dose-related increases in extraneuronal 5-HT that are rapid in onset and decline relatively quickly to basal levels. Thus, the profiles of D-fenfluramine, phentermine and aminorex differ from that of fluoxetine in terms of rapidity of onset, magnitude of 5-HT response and persistence of effect.

When one considers the K_i values of the drugs investigated in the current study, it is apparent that, with the exception of fluoxetine, there is an inverse relationship between affinity for the transporter and the magnitude of effect on extracellular 5-HT after peripheral administration (cf. Table 1 and Fig. 1). The most potent releasing agent in the current study, D-fenfluramine, with a K_i of 280 nM, increases extracellular 5-HT by ~ 1500%. Aminorex (K_i = 1380 nM) is slightly less potent with a maximal increase of ~ 1200%, and phentermine, with the lowest affinity (K_i = 11,200 nM) produces the smallest increase of these drugs (~ 500%). However, even phentermine, with its low affinity for the 5-HT transporter, is approximately twice as efficacious in increasing extracellular 5-HT levels in comparison to the reuptake inhibitor, fluoxetine, with its high affinity for the site (K_i = 11 nM, maximal increase in extracellular 5-HT levels ~ 200%). Thus, although the reuptake inhibitor fluoxetine may have relatively high affinity for the 5-HT transporter in vivo, its effects on 5-HT are small compared with those of the releasing agents. Pharmacokinetic properties could play some role in the differences in the time course and magnitude of effect of these drugs. However, as discussed below, the results of our other experiments demonstrate that these observations are characteristic of the different mechanisms by which reuptake inhibitors and releasing agents elicit changes in 5-HT efflux.

Similarities between D-fenfluramine, phentermine and aminorex, and a difference from fluoxetine were also evident in the results from the local infusion experiment. With local infusion, possible differences in pharmcokinetics such as peripheral metabolism, plasma protein binding or transport into the brain are avoided but fluoxetine still had a relatively small, slowly peaking and sustained effect on 5-HT efflux. Thus, reverse dialysis infusion of fluoxetine (10 μM) increased extraneuronal 5-HT by ~ 7-fold, with levels reaching a plateau at 3 h. Evidence from other studies, including our own, demonstrates that this effect cannot be potentiated even with reverse dialysis infusion of fluoxetine and other selective 5-HT reuptake inhibitors at a concentration of 1 mM (Hervás et al., 2000; Tao et al., 2000). In contrast, reverse dialysis of 100 μM D-fenfluramine, aminorex or phentermine evoked maximal increases in extracellular 5-HT within 1.5 h, and for D-fenfluramine and aminorex, the maximal increases were ~ 32 × and ~ 20 ×, respectively; only phentermine produced a moderate ~ 5-fold increase in 5-HT efflux. Moreover, previous experiments have reported that fenfluramine is capable of potentiating 5-HT efflux >100-fold above baseline (Schwartz et al., 1989). Thus, there are pronounced differences in the time course and maximal efflux elicited by local infusion of a known 5-HT releasing agent compared to a reuptake inhibitor that are independent of pharmacokinetic factors that may influence responses to systemic administration.

Another difference is the apparent dependency on neuronal activity of reuptake blocker-elicited 5-HT efflux. Thus, the 5-HT_{1A} autoreceptor agonist 8-OH-DPAT blocked the increase in extracellular 5-HT evoked by fluoxetine, but not by D-fenfluramine, aminorex or phentermine. This finding supports the view that the actions of reuptake inhibitors are tonically modulated by serotonergic neuronal firing (Gartside et al., 1995) and autoreceptor activation largely offsets the potentiating effect of blocking synaptic clearance (Hjorth, 1993). These results also provide an important confirmation of our earlier finding that 8-OH-DPAT attenuates the increases in hypothalamic extracellular 5-HT evoked by various reuptake inhibitors, but not by the releasing drug, DL-fenfluramine (Gundlah et al., 1997).

At the very low dose we used, 8-OH-DPAT strongly inhibits serotonergic neuronal discharge without major effects on postsynaptic 5-HT_{1A} receptors (Goodwin et al., 1987). Thus, even though D-fenfluramine, aminorex and phentermine had much larger effects compared to fluoxetine, the observation that 5-HT efflux elicited by these drugs was unaltered by 8-OH-DPAT suggests that this effect was independent of depolarization-induced exocytosis. Moreover, because 8-OH-DPAT was administered at a very low dose, it is unlikely that the reversal of fluoxetine's effect could be related to pharmacokinetic interactions. In support of this assumption, results of other studies show that 8-OH-DPAT reverses the increase in 5-HT induced by fluoxetine but not fenfluramine even when administered after drug-induced efflux was maximal (Perry and Fuller, 1992; Rutter et al., 1995; Gundlah et al., 1997). In contrast, in vitro 5-HT efflux elicited by fenfluramine was partly calcium-dependant (Frittoli et al., 1994). Presumably, 5-HT released by exocytosis and fenfluramine directly compete for binding to the transporter. Thus, increased efflux in response to fenfluramine is in part also a result of reuptake inhibition. Nevertheless, the failure of 8-OH-DPAT to alter the effects of fenfluramine, aminorex and phentermine in our microdialysis experiments provides evidence that these drugs primarily elicit 5-HT efflux in vivo by a releasing mechanism.

Related to these observations, systemic administration of fluoxetine during infusion of the reuptake inhibitor into the hypothalamus produced a decrease in extracellular levels of 5-HT. This decrease is the result of inhibition of 5-HT release as a consequence of indirect activation of somato-

dendritic autoreceptors. This conclusion is based on previous studies using this paradigm, which showed that the decrease in extracellular 5-HT is blocked by WAY100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexane-carboxamide), an antagonist of somatodendritic 5-HT_{1A} autoreceptors (Hjorth and Auerbach, 1994; Rutter et al., 1995). In similar 'local-peripheral' experiments, extracellular levels of 5-HT were not decreased in response to systemic administration of D-fenfluramine, aminorex or phentermine, and this also suggests that these three drugs are enhancing 5-HT efflux by a mechanism that is independent of normal neurotransmitter exocytosis.

Considering these results in the light of the criteria we have previously defined for differentiating 5-HT reuptake inhibitors from releasing agents (Gundlah et al., 1997), this confirms that fluoxetine acts solely through reuptake inhibition, whereas D-fenfluramine, phentermine and aminorex demonstrate the characteristics of releasing agents. D-Fenfluramine and aminorex have both been shown to release [^{3}H]5-HT from rat cortical slices in vitro (Table 1; also Heal et al., 1998), and recently, D-fenfluramine has been found to be similarly effective in a rat hypothalamic slice preparation (Prow et al., 2001). The observation that their potentiating effects on 5-HT efflux in vivo can be prevented by blockade of 5-HT reuptake sites with fluoxetine therefore supports the hypothesis that D-fenfluramine and aminorex are entering the presynaptic terminal via this high affinity transporter system and then releasing 5-HT nonphysiologically by displacement. Phentermine, on the other hand, does not evoke release of [^{3}H]5-HT in vitro from rat cortical or hypothalamic slices (see Table 1; Prow et al., 2001). Moreover, it has recently been reported that, in contrast to D-fenfluramine, phentermine is unable to release endogenous platelet 5-HT from rat whole blood in vitro at concentrations ≤ 300 μM (Prow et al., 2000). On the basis of these results, it would be predicted that blockade of the 5-HT reuptake carrier would not attenuate phentermine's potentiating effect on 5-HT efflux in vivo. However, as the data clearly demonstrate, reuptake blockade by fluoxetine prevents phentermine's effect on 5-HT as completely as that of D-fenfluramine or aminorex. This discrepancy between in vivo and in vitro actions may be explained by the possibility that phentermine's releasing action is selective for the newly synthesised 5-HT "releasable pool", rather than the "storage pool" that is predominantly labeled by preloading with [^{3}H]5-HT. This idea is supported by the recent publication of Rothman et al. (2001), which reported that phentermine did evoke release of [^{3}H]5-HT from synaptosomes following reserpinization.

It is possible that efflux of 5-HT elicited by some releasing agents may be mediated either secondary to dopamine release as suggested in the case of phentermine (see Balcioglu and Wurtman, 1998b) or through inhibition of monoamine oxidase (see Ulus et al., 2000). However, because blockade of the 5-HT reuptake transporter abolished 5-HT efflux evoked by all three releasing agents, it appears that these drugs interact directly at the 5-HT reuptake site. Further support for this conclusion is the evidence that dopamine receptor agonists exert comparatively small effects on extracellular 5-HT levels (Ferré and Artigas, 1993; Mendlin et al., 1998; Martin-Ruiz et al., 2001), and the weak, reversible inhibition of monoamine $oxidase_A$ by D-fenfluramine, D-norfenfluramine and phentermine is unlikely to be of any pharmacological relevance (Kilpatrick et al., 2001).

The final aim of this investigation was to determine the effects of the phen/fen and phen/fluoxetine combination under conditions most relevant to the control of food intake. When tested in the dark phase of the light/dark cycle, we observed an additive effect of phentermine and D-fenfluramine on 5-HT efflux, and this is consistent with a recent report for the interaction of these two drugs on hypothalamic 5-HT determined during the light phase (Prow et al., 2001). These results are, therefore, in limited agreement with data from other microdialysis experiments conducted in the striatum and nucleus accumbens that have observed either a small or no effect of phentermine on 5-HT (Shoaib et al., 1997; Balcioglu and Wurtman, 1998b; Baumann et al., 2000), although it is accepted that this may partly be a reflection of differences in the doses employed in the various studies. In terms of the additive, and possibly synergistic, effect of the phen/fen combination on food intake in rats (Rowland et al., 2000) and on human obesity (Weintraub, 1992), the potential mechanisms underpinning this phenomenon are complex. Noradrenaline and dopamine, as well as 5-HT, play key roles in the regulation of energy expenditure (see reviews of Sugrue, 1987; Silverstone, 1992) and it is apparent that, at higher pharmacologically relevant doses, the actions of phentermine and D-fenfluramine are not restricted to noradrenaline and 5-HT, respectively, but extend to dopamine (Shoaib et al., 1997; Balcioglu and Wurtman, 1998a,b; Rothman et al., 1999; Baumann et al., 2000). This indicates that the phen/fen combination works through a multiplicity of monoaminergic systems. Finally, in the search for more effective anti-obesity drug therapies, various combinations of monoaminergic drugs have been suggested (see Atkinson et al., 1995), and recently, there have been suggestions that phentermine could be combined with fluoxetine (Prozac®) (phen/Pro). The data indicate that effect of fluoxetine on food intake is comparatively weak (Jackson et al., 1997; Rowland et al., 2000) and this selective 5-HT reuptake inhibitor does not produce sustained weight-loss in patients (Goldstein et al., 1994). This is explicable on the basis of fluoxetine's selectivity for blocking 5-HT reuptake and relatively small impact on extracellular 5-HT in the hypothalamus when compared with 5-HT releasing agents (Gundlah et al., 1997; Heal et al., 1998; this study). Whilst the results from the current investigation do not preclude the opportunity for an additive or synergistic interaction between phentermine and fluoxetine based on noradrenergic or dopaminergic mechanisms, they nevertheless indicate

that any potential contribution from phentermine that is mediated via enhanced 5-HT function is likely to be attenuated by giving these two drugs in combination. This contrasts with the phen/fen combination where their actions on central 5-HT function are at least additive.

In conclusion, this study has shown that phentermine and aminorex, which were originally considered to be catecholaminergic drugs, markedly increase extraneuronal 5-HT levels in rat hypothalamus. Their actions are less profound than D-fenfluramine, but are considerably greater than that of the selective 5-HT reuptake inhibitor, fluoxetine. Pharmacological characterization reveals that D-fenfluramine, aminorex and probably also phentermine potentiate synaptic 5-HT function predominantly by release, whereas for fluoxetine, the mechanism is exclusively reuptake inhibition. The results also show that the combined actions of phentermine and D-fenfluramine on extracellular 5-HT levels in the rat hypothalamus are additive and not synergistic. In contrast, there is significant negative cooperativity on 5-HT efflux of using phentermine and fluoxetine in combination.

References

Abenhaim, L., Moride, Y., Brenot, F., Rich, S., Benichou, J., Kurz, X., Higenbottam, T., Oakley, C., Wouters, E., Aubier, M., Simonneau, G., Begaud, B., 1996. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. N. Engl. J. Med. 335, 609–616.

Atkinson, R.L., Blank, R.C., Loper, J.F., Schumacher, D., Lutes, R.A., 1995. Combined drug treatment of obesity. Obes. Res. 3, 475S–500S.

Auerbach, S.B., Minzenberg, M.J., Wilkinson, L.O., 1989. Extracellular serotonin and 5-hydroxyindoleacetic acid in hypothalamus of the unanesthetized rat measured by in vivo dialysis coupled to high-performance liquid chromatography with electrochemical detection: dialysate serotonin reflects neuronal release. Brain Res. 499, 281–290.

Balcioglu, A., Wurtman, R.J., 1998a. Effects of phentermine on striatal dopamine and serotonin release in conscious rats: in vivo microdialysis study. Int. J. Obes. Relat. Metab. Disord. 22, 325–328.

Balcioglu, A., Wurtman, R.J., 1998b. Effects of fenfluramine and phentermine (fen–phen) on dopamine and serotonin release in rat striatum: in vivo microdialysis study in conscious animals. Brain Res. 813, 67–72.

Baumann, M.H., Ayestas, M.A., Dersch, C.M., Brockington, A., Rice, K.C., Rothman, R.B., 2000. Effects of phentermine and fenfluramine on extracellular dopamine and serotonin in rat nucleus accumbens: therapeutic implications. Synapse 36, 102–113.

Blundell, J.E., Halford, J.C.G., 1998. Serotonin and appetite regulation. Implications for the pharmacological treatment of obesity. CNS Drugs 9, 473–495.

Borsini, F., Bendotti, C., Samanin, R., 1985. Salbutamol, D-amphetamine and D-fenfluramine reduce sucrose intake in freely fed rats by acting on different neurochemical mechanisms. Int. J. Obes. 9, 277–283.

Boulos, A., Terman, M., 1979. Splitting of circadian rhythms in the rat. J. Comp. Physiol. 134, 75–83.

Caccia, S., Bizzi, A., Coltro, G., Fracasso, C., Frittoli, E., Mennini, T., Garatinni, S., 1992. Anorectic activity of fluoxetine and norfenfluramine in rats: relationship between brain concentrations and in vitro potencies on monoaminergic mechanisms. J. Pharm. Pharmacol. 44, 250–254.

Cheetham, S.C., Viggers, J.A., Slater, N.A., Heal, D.J., 2000. Sibutramine does not decrease the number of 5-HT reuptake sites in the brain and, like fluoxetine, protects against the deficits produced by dexfenfluramine. Neuropharmacology 39, 2028–2035.

Clifton, P.G., Barnfield, A.M.C., Philcox, L., 1989. A behavioural profile of fluoxetine-induced anorexia. Psychopharmacology 97, 89–95.

Connolly, H.M., Crary, J.L., McGoon, M.D., Hensrud, D.D., Edwards, B.S., Edwards, W.D., Schaff, H.V., 1997. Valvular heart disease associated with fenfluramine–phentermine. N. Engl. J. Med. 9, 581–588.

Dailey, J.W., Yan, Q.S., Mishra, P.K., Burger, R.L., Jobe, P.C., 1992. Effects of fluoxetine on convulsions and on brain serotonin as detected by microdialysis in genetically epilepsy-prone rats. J. Pharmacol. Exp. Ther. 260, 533–540.

Ferré, S., Artigas, F., 1993. Dopamine D2 receptor-mediated regulation of serotonin extracellular concentration in the dorsal raphe nucleus of freely moving rats. J. Neurochem. 61, 772–775.

Frittoli, E., Gobbi, M., Mennini, T., 1994. Involvement of p-type Ca^{2+} channels in the K^{+}- and D-fenfluramine-induced [H-3]5-HT release from rat hippocampal synaptosomes. Neuropharmacology 33, 833–835.

Garabette, M.L., Martin, K.F., Redfern, P.H., 2000. Circadian variation in the activity of the 5-HT_{1B} autoreceptor in the region of the suprachiasmatic nucleus, measured by microdialysis in the conscious freely-moving rat. Br. J. Pharmacol. 131, 1569–1576.

Gartside, S.E., Umbers, V., Hajós, M., Sharp, T., 1995. Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. Br. J. Pharmacol. 115, 1064–1070.

Goldstein, D.J., Rampey, A.H., Enas, G.G., Potvin, J.H., Fludzinski, L.A., Levine, L.R., 1994. Fluoxetine: a randomized clinical trial in the treatment of obesity. Int. J. Obes. 18, 129–135.

Goodwin, G.M., De Souza, R.J., Green, A.R., Heal, D.J., 1987. The pharmacology of the behavioural and hypothermic responses of rats to 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Psychopharmacology 91, 506–511.

Grignaschi, G., Samanin, R., 1993. Role of serotonin receptors in the effect of sertraline on feeding behaviour. Psychopharmacology 110, 203–208.

Gundlah, C., Martin, K.F., Heal, D.J., Auerbach, S.B., 1997. In vivo criteria to differentiate monoamine reuptake inhibitors from releasing agents: sibutramine is a reuptake inhibitor. J. Pharmacol. Exp. Ther. 283, 581–591.

Heal, D.J., Cheetham, S.C., Prow, M.R., Martin, K.F., Buckett, W.R., 1998. A comparison of the effects on central 5-HT function of sibutramine hydrochloride and other weight modifying agents. Br. J. Pharmacol. 125, 301–308.

Hervás, I., Queiroz, C.M., Adell, A., Artigas, F., 2000. Role of uptake inhibition and autoreceptor activation in the control of 5-HT release in the frontal cortex and dorsal hippocampus of the rat. Br. J. Pharmacol. 130, 160–166.

Hjorth, S., 1993. Serotonin 5-HT_{1A} autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo—a microdialysis study. J. Neurochem. 60, 776–779.

Hjorth, S., Auerbach, S.B., 1994. Further evidence for the importance of 5-HT_{1A} autoreceptors in the action of selective serotonin reuptake inhibitors. Eur. J. Pharmacol. 260, 251–255.

Jackson, H.C., Needham, A.M., Hutchins, L.J., Mazurkiewicz, S.E., Heal, D.J., 1997. Comparison of the effects of sibutramine and other monoamine reuptake inhibitors on food intake in the rat. Br. J. Pharmacol. 121, 1758–1762.

Kilpatrick, I.C., Traut, M., Heal, D.J., 2001. Monoamine oxidase inhibition is unlikely to be relevant to the risks associated with phentermine and fenfluramine: a comparison with their abilities to evoke monoamine release. Int. J. Obes. 25, 1454–1458.

Lancashire, B., Viggers, J., Prow, M.R., Aspley, S., Martin, K.F., Cheetham, S.C., Heal, D.J., 1998. Monoamine release and uptake inhibition profiles of antiobesity agents. J. Psychopharmacol. 12 (Suppl. A), A36.

Lee, M.D., Clifton, P.G., 1992. Partial reversal of fluoxetine anorexia by the 5-HT antagonist metergoline. Psychopharmacology 107, 359–364.

Leibowitz, S.F., Alexander, J.T., 1998. Hypothalamic serotonin in control

of eating behaviour, meal size and body weight. Biol. Psychiatry 44, 851–864.
Lew, R., Weisenberg, B., Vosmer, G., Seiden, L.S., 1997. Combined phentermine/fenfluramine administration enhances depletion of serotonin from central terminal fields. Synapse 26, 36–45.
Lucki, I., Kreider, M.S., Simansky, K.J., 1988. Reduction of feeding behaviour by the serotonin uptake inhibitor sertraline. Psychopharmacology 96, 289–295.
Malagié, I., Trillat, A.-C., Jacquot, C., Gardier, A.M., 1995. Effects of acute fluoxetine on extracellular serotonin levels in the raphe: an in vivo microdialysis study. Eur. J. Pharmacol. 286, 213–217.
Martin, K.F., Marsden, C.A., 1985. In vivo diurnal variations of 5HT release in hypothalamic nuclei. In: Redfern, P.H., Campbell, I.C., Davies, J.A., Martin, K.F. (Eds.), Circadian Rhythms in the Central Nervous System. Macmillan, London, pp. 81–92.
Martin, K.F., Redferm, P.H., 1997. 5-Hydroxytryptamine and noradrenaline synthesis, release and metabolism in the central nervous system: circadian rhythms and control mechanisms. In: Redfern, P.H., Lemmer, B. (Eds.), Handbook of Experimental Pharmacology, Physiology and Pharmacology of Biological Rhythms vol. 125, pp. 157–176.
Martin-Ruiz, R., Ugedo, L., Honrubia, M.A., Mengod, G., Artigas, F., 2001. Control of serotonergic neurons in rat brain by dopaminergic receptors outside the dorsal raphe nucleus. J. Neurochem. 77, 762–775.
McCann, U., Hatzidimitriou, G., Ridenour, A., Fischer, C., Yuan, J., Katz, J., Ricaurte, G., 1994. Dexfenfluramine and serotonin neurotoxicity: further preclinical evidence that clinical caution is indicated. J. Pharmacol. Exp. Ther. 269, 792–798.
McCann, U.D., Yuan, J., Ricaurte, G.A., 1998. Neurotoxic effects of (±)fenfluramine and phentermine, alone and in combination, on monoamine neurons in the mouse brain. Synapse 30, 239–246.
McNeely, W., Goa, K.L., 1998. Sibutramine. A review of its contribution to the management of obesity. Drugs 56, 1093–1124.
Mendlin, A., Martin, F.J., Jacobs, B.L., 1998. Involvement of dopamine D_2 receptors in apomorphine-induced facilitation of forebrain serotonin output. Eur. J. Pharmacol. 351, 291–298.
Molliver, D.C., Molliver, M.E., 1990. Anatomic evidence for a neurotoxic effect of (+/−)-fenfluramine upon serotonergic projections in the rat. Brain Res. 511, 165–168.
Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd edition. Academic Press, Sydney.
Perry, K.W., Fuller, R.W., 1992. Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. Life Sci. 50, 1683–1690.
Prow, M.R., Lane, E.L., Aspley, S., Kilpatrick, I.C., Heal, D.J., 2000. D-Fenfluramine releases 5-HT from rat whole blood as measured by in vitro microdialysis. Int. J. Obes. 24 (Suppl. 1), 410.
Prow, M.R., Lancashire, B., Aspley, S., Heal, D.J., Kilpatrick, I.C., 2001. Additive effects on rat brain 5-HT release of combining phentermine with dexfenfluramine. Int. J. Obes. 25, 1450–1453.
Rothman, R.B., Ayestas, M.A., Dersch, C.M., Baumann, M.H., 1999. Aminorex, fenfluramine, and chlorphentermine are serotonin transporter substrates. Implications for primary pulmonary hypertension. Circulation 100, 869–875.
Rothman, R.B., Baumann, M.H., Dersch, C.M., Romero, D.V., Rice, K.C., Carroll, F.I., Partilla, J.S., 2001. Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39, 32–41.
Rowland, N.E., Carlton, J., 1988. Dexfenfluramine: effects on food intake in various animal models. Clin. Neuropharmacol. 11, S33–S50.
Rowland, N.E., Marshal, M., Roth, J.D., 2000. Comparison of either norepinephrine-uptake inhibitors or phentermine combined with serotonergic agents on food intake in rats. Psychopharmacology 149, 77–83.
Rutter, J.J., Gundlah, C., Auerbach, S.B., 1995. Systemic uptake inhibition decreases serotonin release via somatodendritic autoreceptor activation. Synapse 20, 225–233.
Samanin, R., Mennini, T., Bendotti, C., Barone, D., Caccia, S., Garattini, S., 1989. Evidence that central 5-HT_2 receptors do not play an important role in the anorectic activity of D-fenfluramine in the rat. Neuropharmacology 28, 465–469.
Sayer, T.J.O., Hannon, S.D., Redfern, P.H., Martin, K.F., 1999. Diurnal variation in 5-HT_{1B} autoreceptor function in the anterior hypothalamus in vivo: effect of chronic antidepressant drug treatment. Br. J. Pharmacol. 126, 1777–1784.
Schwartz, D., Hernandez, L., Hoebel, B.G., 1989. Fenfluramine administered systemically or locally increases extracellular serotonin in the lateral hypothalamus as measured by microdialysis. Brain Res. 482, 261–270.
Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. Nature 404, 661–671.
Shoaib, M., Baumann, M.H., Rothman, R.B., Goldberg, S.R., Schindler, C.W., 1997. Behavioural and neurochemical characteristics of phentermine and fenfluramine administered separately and as a mixture in rats. Psychopharmacology 131, 296–306.
Silverstone, T., 1992. Appetite suppressants. A review. Drugs 43, 820–836.
Sugrue, M.F., 1987. Neuropharmacology of drugs affecting food intake. Pharmacol. Ther. 32, 145–182.
Tao, R., Ma, Z., Auerbach, S.B., 2000. Differential effect of local infusion of serotonin reuptake inhibitors in the raphe versus forebrain and the role of depolarization-induced release in increased extracellular serotonin. Br. J. Pharmacol. 294, 571–579.
Ulus, I.H., Maher, T.J., Wurtman, R.J., 2000. Characterization of phentermine and related compounds as monoamine oxidase (MAO) inhibitors. Biochem. Pharmacol. 59, 1611–1621.
Weintraub, M., 1992. Long-term weight control study: conclusions. Clin. Pharmacol. Ther. 51, 642–646.
Williams, G., Harrold, J.A., Cutler, D.J., 2000. The hypothalamus and the regulation of energy homeostasis: lifting the lid on a black box. Proc. Nutr. Soc. 59, 385–396.
Wortley, K.E., Heal, D.J., Stanford, S.C., 1999. Modulation of sibutramine-induced increases in extracellular noradrenaline concentration in rat frontal cortex and hypothalamus by α_2-adrenoceptors. Br. J. Pharmacol. 128, 659–666.
Zheng, Y., Russell, B., Schmierer, D., Laverty, R., 1997. The effects of aminorex and related compounds on brain monoamines and metabolites in CBA mice. J. Pharm. Pharmacol. 49, 89–96.