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Comparison of the thermogenic and hypophagic effects of sibutramine's metabolite 2 and other monoamine reuptake inhibitors

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Abstract

The thermogenic and hypophagic effects of sibutramine's metabolite (metabolite 2), a 5-hydroxytryptamine (5-HT) and noradrenaline reuptake inhibitor, were investigated in rats and compared with duloxetine and bupropion. Metabolite 2 increased colonic temperature for 2.5–4.5 h. Duloxetine was also thermogenic but was less effective than metabolite 2. Bupropion similarly increased colonic temperature and was as efficacious, but less potent, than metabolite 2. At −8 °C, metabolite 2, duloxetine and bupropion all decreased response to heat reinforcement and reduced colonic temperature. Metabolite 2 produced a sustained increase in oxygen consumption (VO_2) at 29 °C from 90 to 240 min, whereas duloxetine was far less effective. Bupropion rapidly enhanced VO_2, but its effect was less prolonged than that of metabolite 2. Metabolite 2 markedly reduced 24-h food intake. Duloxetine decreased feeding although its effect was shorter-lived, but bupropion was without effect. Thus, sibutramine's antiobesity action is probably attributable to effects on energy intake and expenditure. Duloxetine shares these properties, but is generally less efficacious. Any potential weight-reducing effect of bupropion is probably due to thermogenesis.

Keywords: Sibutramine; BTS 54 505; Duloxetine; Bupropion; Thermogenesis; Food intake

1. Introduction

Sibutramine hydrochloride monohydrate (BTS 54 524, *N*-{1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl}-*N*,*N*-dimethylamine HCl monohydrate, Meridia®Reductil®) is a centrally acting drug that has received global approval for weight reduction and maintenance in obesity. In vitro, sibutramine is a weak reuptake inhibitor of noradrenaline in rat brain and 5-HT in human brain (Heal et al., 1998). However, its secondary amine metabolite (metabolite 1, BTS 54 354, *N*-[1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutyl]-*N*-methylamine HCl) and primary amine metabolite (metabolite 2, BTS 54 505, 1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine HCl) are potent in vitro reuptake inhibitors of both monoamines (Luscombe et al., 1989; Cheetham et al., 1993, 1996; Heal et al., 1998) and also of dopamine in vitro (Luscombe et al., 1989; Heal et al., 1998). At therapeutically relevant doses in vivo, however, sibutramine's actions are limited to reuptake inhibition of noradrenaline and 5-HT in animals (Heal et al., 1992; Rowley et al., 2000) and in man (Luscombe et al., 1990). With this pharmacological profile, it is not surprising to learn that sibutramine was discovered and developed initially as a potentially advantaged antidepressant drug (Buckett et al., 1988). Whilst the efficacy of sibutramine was not proven in two large Phase II clinical trials in depression, consistent weight loss was observed in these patients (Kelly et al., 1995) which led to the subsequent successful development of sibutramine as an antiobesity drug. Preclinical investigations in rodents indicate that sibutramine decreases bodyweight both by reducing food intake (Jackson et al., 1997a,b) via enhanced satiety (Halford et al., 1995), and by increasing energy expenditure via enhanced resting metabolic rate (Connoley et al., 1999). Both mechanisms are central in origin (Heal et al., 1998; Connoley et al., 1999)

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and result from a synergistic interaction between noradrenergic and 5-hydroxytryptaminergic neuronal systems in the brain (Jackson et al., 1997b; Connoley et al., 1999). The overlap between the central monoaminergic mechanisms responsible for antidepressant and weight loss efficacy is well known; an example being the serotonin selective reuptake inhibitor antidepressant, fluoxetine, which has been evaluated in the treatment of obesity where it was found to evoke clinically meaningful weight loss (Ferguson and Feighner, 1987; Levine et al., 1989), but this effect was not maintained at 1 year (Goldstein et al., 1994).

Duloxetine is another reuptake inhibitor of noradrenaline and 5-HT (Wong et al., 1993) that has been reported to demonstrate antidepressant-like activity in a wide range of preclinical models (Katoh et al., 1995). These include reversing or preventing the actions of the monoamine depleting agents, reserpine and tetrabenazine, and reducing immobility in the forced-swimming test (Katoh et al., 1995). Intracerebral microdialysis experiments have demonstrated that acute administration of duloxetine elevates the extraneuronal concentration of 5-HT and noradrenaline in the rat frontal cortex and hypothalamus, respectively, indicating that this drug is a functional reuptake inhibitor of both monoamines in vivo (Engleman et al., 1995; Kihara and Ikeda, 1995). Whilst there are some acute studies that have shown duloxetine reduces food intake when given acutely (Wong et al., 1993; Jackson et al., 1997b), the action of this compound on metabolic rate has not so far been explored. Bupropion is also a monoamine reuptake inhibitor, but it differs from sibutramine and duloxetine because this drug is a weak reuptake inhibitor that shows ~2.5-fold selectivity for dopamine versus noradrenaline, and it is inactive against 5-HT (Hyttel, 1982; Richelson and Pfenning, 1984). Bupropion is approved for use both as an antidepressant (Wellbutrin®) (Ascher et al., 1995) and for smoking cessation (Zyban®) (Hughes, 2000). Interestingly, it has been shown that subjects receiving bupropion gained significantly less weight than placebo-treated patients (Harto-Truax et al., 1983; Jorenby et al., 1999) suggesting that this drug may alter energy balance. Despite this fact, bupropion was not found to decrease either appetite or calorie intake in man (Miller and Griffith, 1983), although it has been shown to reduce food intake in rats, but only at behaviourally activating doses (Zarrindast and Hosseini-Nia, 1988). Once again, there have been no studies conducted to explore bupropion's potential effects on thermogenesis in animals. Thus, in the present study, we have investigated the effects of both duloxetine and bupropion on thermogenesis by measuring oxygen consumption, body temperature and thermoregulatory behaviour and, in addition, determined their effects on food intake. These effects have been compared with those of sibutramine's primary amine metabolite, metabolite 2; this compound was chosen because its thermogenic actions are more rapid in onset than those of sibutramine (Connoley et al., 1999).

2. Materials and methods

2.1. Animals

Experiments were performed on female Wistar rats (200–250 g at the start of the experiment), which were obtained from the colony maintained at St George's Hospital Medical School. Animals were housed in pairs (and individually for the oxygen consumption and thermoregulatory behaviour experiments) at a room temperature of 21 ± 1 °C. They were maintained on a conventional pelleted stock diet with free access to water with a 12-h light cycle (lights on 0700 h).

2.1.1. Chemicals

Sibutramine's primary amine metabolite, metabolite 2 (BTS 54 505; 1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine HCl; Knoll Research and Development, Nottingham), was dissolved in sterile saline, duloxetine (Lilly) was suspended in 0.25% cellulose and bupropion HCl (Research Biochemicals International, St. Albans, UK) was dissolved in deionized water. All drugs were administered orally (gavage) using a dose volume of 5 ml/kg.

2.2. Measurement of food intake and body temperature

Animals were acclimatised to the experimental conditions, e.g. being handled and colonic temperature measurement, for at least 1 week before the experiments were conducted. On the test day, animals were randomised to treatment groups. Food hoppers were weighed just before drug administration (09.00 h) and 4.5, 8.0 and 24.0 h thereafter. Colonic temperature (T_c) was measured using a thermocouple probe (Physiotemp, model BAT-12) inserted approximately 6 cm into the rectum and held until a steady temperature had been recorded. The pretrained rats were allowed to sit unrestrained on the bench during this procedure. Measurements were made at time 0.0 h (just before giving drugs), and then at 0.5, 1.0, 1.5, 2.5, 3.5 and 4.5 h post-drug administration.

2.3. Measurement of thermoregulatory behavioural responsiveness—operant response to exogenous heat

The test apparatus consisted of a circular 23-cm diameter and 24-cm deep wire-mesh cage. A 4 × 5-cm plexiglas lever mounted on a microswitch protruded 5 cm into the cage and 2 cm above the floor. Two 250-W red bulb infrared lamps were placed at each side of the cage at a 45° angle to the floor and focussed on the rat at the lever. The power dissipated by the lamps was set to 300 W, which produced an irradiance of 180 mW/cm^2. The apparatus was placed in a freezer maintained at −8 ± 2 °C. A 25-W red incandescent lamp provided low-level background illumination. The heat lamps were

activated by pressing the lever and remained on as long as the lever was held down. The number of lever presses and the cumulative duration of heat lamp activation (as seconds of heat) were recorded on a computer linked to the apparatus (Carlisle and Stock, 1991). The animals were shaved closely on the day before the test. They were first trained to press the lever for radiant heat and then given at least six additional tests of 120-min duration so that performance was stable and consistent for two consecutive tests. On the day of the experiment, four animals were brought to the laboratory in the fed state. They were randomised into two treatment groups (vehicle and drug-treated). After 30 min, rats were taken out and their colonic temperatures (“pretest”) were recorded. They were then orally dosed with either vehicle or drugs and put back into the freezer for the bar-press test. After 60 min, rats were taken out and their colonic temperatures were again measured (“posttest”).

2.4. Measurement of oxygen consumption

Oxygen consumption (VO_2) was determined in closed-circuit respirometers maintained at the thermoneutral temperature for rats (29 °C). The system allows for eight rats to be individually tested at one time (Stock, 1975). All animals were accustomed to the respirometers and procedures on two occasions the week before the experiments. Oxygen consumption was recorded on a computer every 5 min and expressed as milliliter of oxygen per kilogram of metabolic body size per minute (i.e. ml/$kg^{0.75}$/min). After a 90-min measurement of baseline VO_2, animals were removed and colonic temperatures were measured. Rats were then given either vehicle or drugs before being returned to the chambers. Measurement of VO_2 was continued for a further 4-h posttreatment with drugs. The 30-min averages of VO_2 were used to plot the time-course of the responses and the increase in VO_2 was determined by comparing the mean of the last 30 min of the baseline readings with the mean values obtained for the posttreatment period.

2.5. Statistics

Results have been presented as means ± S.E.M. Statistical comparisons of the different treatment groups were made by Student’s *t*-test for paired data. One-way analysis of variance followed by the Dunnett’s test was used for multiple comparisons of differences from a single control. All probabilities quoted are two-tailed, with $P<0.05$ being taken as the level of significance.

3. Results

The respective effects of metabolite 2 (10 mg/kg, p.o.) and duloxetine (10, 20 and 30 mg/kg, p.o.) on colonic temperature and food intake are shown in Fig. 1. Colonic temperature in vehicle-treated animals declined gradually over the 4.5-h period after dosing from 38.5 to 37.2 °C (Fig. 1A). Treatment with metabolite 2 (10 mg/kg, p.o.) resulted in a significantly higher colonic temperature (sustained at above basal level) at 2.5, 3.5 and 4.5 h posttreatment ($P<0.01$) compared to that of vehicle-treated animals (Fig. 1A). Duloxetine did not sustain colonic temperature. However, the fall in colonic temperature of the duloxetine (20 and 30 mg/kg, p.o.)-treated rats was much smaller than in the vehicle-treated controls, resulting in significant elevations at 2.5 h (20 and 30 mg/kg, p.o.) and 3.5 h (20 mg/kg, p.o.) post-drug administration ($P<0.05$). There was no significant difference between vehicle- and duloxetine-treated groups at 4.5 h. Duloxetine (10 mg/kg, p.o.) had no significant effect on colonic temperature at any time-point measured (Fig. 1A).

Treatment with metabolite 2 (10 mg/kg, p.o.) also markedly reduced food intake (Fig. 1B). The cumulative food

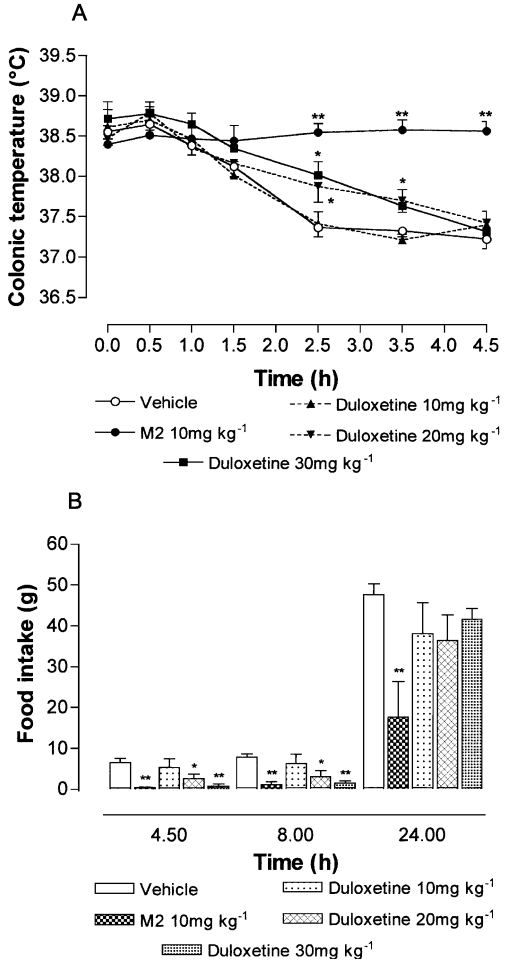

Fig. 1. Effect of metabolite 2 (10 mg/kg, p.o.) and duloxetine (10, 20 and 30 mg/kg, p.o.) on (A) colonic temperature and (B) food intake. Results are means ± S.E.M. ($n=6–12$ for colonic temperature, $n=3–6$ for food intake). $*P<0.05$, $**P<0.01$ vs. vehicle control (Dunnett’s test). The experimental procedures are fully described in Section 2.

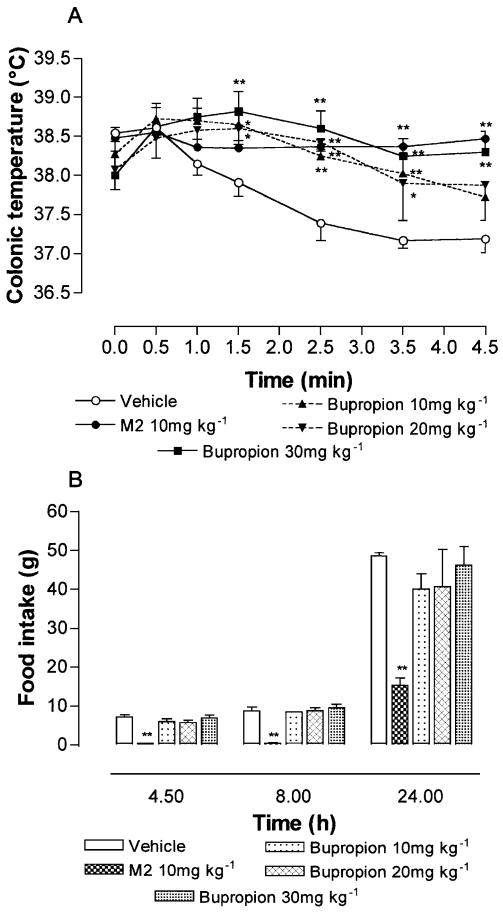

Fig. 2. Effect of metabolite 2 (10 mg/kg, p.o.) and bupropion (10, 20 and 30 mg/kg, p.o.) on (A) colonic temperature and (B) food intake. Results are means ± S.E.M. ($n=8$ for colonic temperature, $n=4$ for food intake). $*P<0.05$, $**P<0.01$ vs. vehicle control (Dunnett's test). The experimental procedures are fully described in Section 2.

intake during the first 8 h period was 1.1 g compared with 7.8 g for the vehicle-treated animals and this hypophagic effect of metabolite 2 was present for ≥24 h after acute dosing ($P<0.01$, Fig. 1B). Duloxetine also decreased food intake, but it was less potent than metabolite 2, e.g. a dose 30 mg/kg, p.o. of duloxetine was equivalent to 10 mg/kg, p.o. of metabolite 2 in reducing food intake. The hypophagic effect of duloxetine (20 and 30 mg/kg, p.o.) was shorter-lived (~ 8 h) when compared with metabolite 2. Unlike metabolite 2, duloxetine had no effect on cumulative food intake over a 24-h period (Fig. 1B).

The effect of bupropion (10, 20 and 30 mg/kg) on colonic temperature and food intake was also compared with that of metabolite 2 (10 mg/kg, p.o., Fig. 2). Metabolite 2 (10 mg/kg, p.o.) significantly increased colonic temperature at 2.5, 3.5 and 4.5 h posttreatment ($P<0.01$) when compared with vehicle-treated controls. Bupropion (10, 20 and 30 mg/kg, p.o.) also significantly increased colonic temperature at 1.5, 2.5 and 3.5 h postadministration. At 4.5 h, the effect of bupropion (10 and 20 mg/kg, p.o.) on colonic temperature no longer reached statistical significance. In contrast, a dose of 30 mg/kg, p.o. bupropion was as efficacious as metabolite 2 (10 mg/kg, p.o.) in elevating colonic temperature ($P<0.01$, Fig. 2A). However, unlike metabolite 2, which markedly reduced food intake over 24 h, bupropion (10, 20 or 30 mg/kg, p.o.) had no effect on cumulative food intake over 24 h (Fig. 2B).

The effect of metabolite 2 (10 mg/kg, p.o.) on thermoregulatory behaviour—operant response to exogenous heat at −8 °C in shaved and previously trained rats—is shown in Fig. 3. Metabolite 2 (10 mg/kg, p.o.) significantly increased bar pressing over the 60-min test period (67% above control value; $P<0.05$, Fig. 3A). However, this did not result in an increase in heat reinforcement. On the contrary, the duration of heat reinforcement (total seconds of heat over 60 min) was decreased by 26% ($P<0.05$, Fig. 3B). The colonic temperature of all of the rats increased during the first 30 min basal run (pretest) and the saline-treated animals maintained their colonic temperature at this level to the end of the 60-min test (posttest), whereas rats receiving metabolite 2 (10 mg/kg,

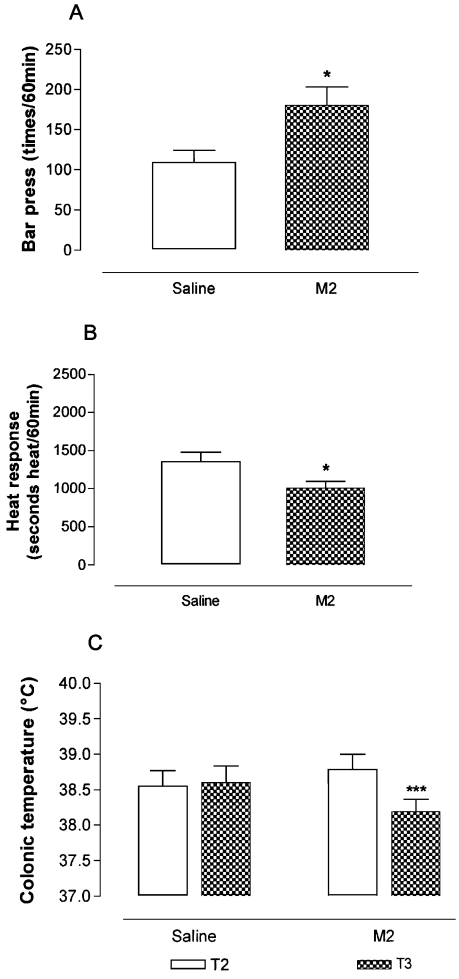

Fig. 3. Effect of metabolite 2 (10 mg/kg, p.o.) on (A) bar-press activity, (B) heat reinforcement at −8° and (C) colonic temperature. Results are means ± S.E.M. ($n=8$). $*P<0.05$, $***P<0.001$ vs. control (Student's paired t-test). The experimental procedures are fully described in Section 2.

p.o.) had significantly lower colonic temperatures at the end of the 60-min test ($P<0.001$, Fig. 3C). Duloxetine (30 mg/kg, p.o.), however, had no effect on the number of bar presses (Fig. 4A), but the duration of heat reinforcement was 23% lower than that of saline-treated animals ($P<0.05$, Fig. 4B). There was also a significant decrease in posttest colonic temperature of the duloxetine-treated rats after 60 min at − 8 °C (Fig. 4C). A response pattern similar to metabolite 2 was seen with bupropion (30 mg/kg, p.o.) in a separate study, where the number of bar presses was significantly higher in bupropion-treated animals ($P<0.01$, Fig. 4A). Bupropion also caused 20% decrease in the total duration of heat reinforcement during the 60-min test ($P<0.05$, Fig. 5B). Posttest colonic temperature was significantly lower than the pretest value for the bupropion-treated animals, whereas there was no such a change in vehicle-treated animals before or after the test (Fig. 5C).

The thermogenic property of duloxetine, bupropion and metabolite 2 were also determined by measuring oxygen

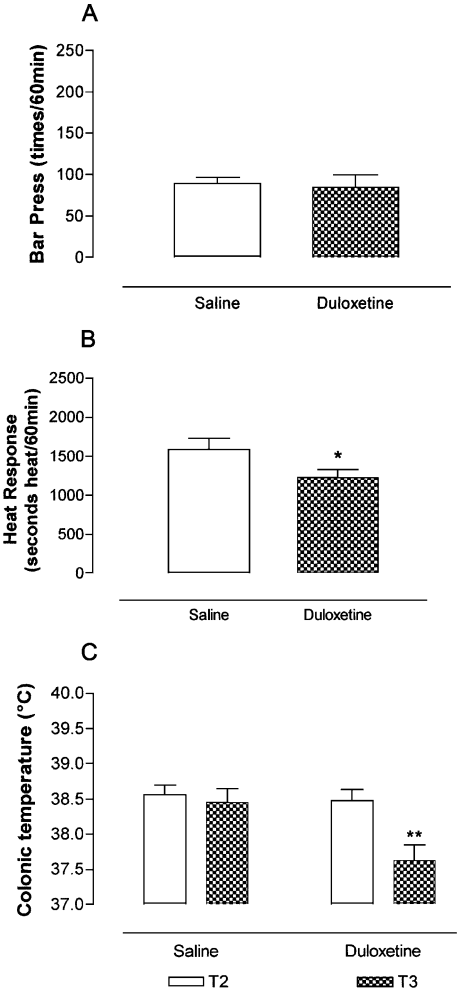

Fig. 4. Effect of duloxetine (30 mg/kg, p.o.) on (A) bar-press activity, (B) heat reinforcement and (C) colonic temperature. Results are means ± S.E.M. ($n=7$). * $P<0.05$, ** $P<0.01$ vs. control (Student's paired t-test). The experimental procedures are fully described in Section 2.

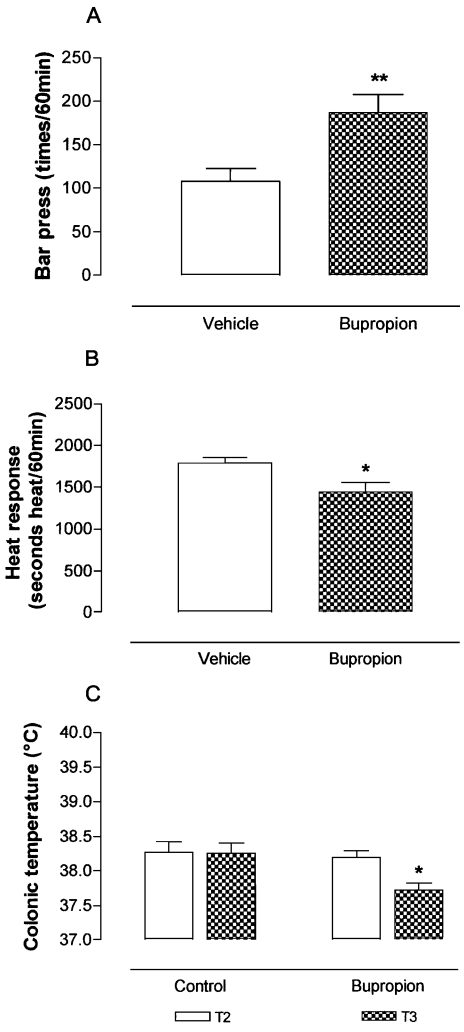

Fig. 5. Effect of bupropion (30 mg/kg, p.o.) on (A) bar-press activity, (B) heat reinforcement and (C) colonic temperature. Results are means ± S.E.M. ($n=6$). * $P<0.05$, ** $P<0.01$ vs. control (Student's paired t-test). The experimental procedures are fully described in Section 2.

consumption (VO_2) at thermoneutrality (29 °C). Results are shown in Fig. 6 as percentage changes from basal values. VO_2 in vehicle-treated animals declined gradually during the course of 4.0 h experiment (basal: 13.49 ± 0.47 ml/kg$^{0.75}$/min; 4 h: 11.67 ± 0.36 ml/kg$^{0.75}$/min, $n=8$). A significant increase in VO_2 was seen at 90 min post-metabolite 2 (30 mg/kg, p.o.) administration and remained elevated during the entire 4 h (basal: 12.98 ± 0.56 ml/kg$^{0.75}$/min; 4 h: 14.51 ± 0.77 ml/kg$^{0.75}$/min, $n=8$). Maximal stimulation of VO_2 by duloxetine (30 mg/kg, p.o.) was seen 30 min ($P<0.05$) posttreatment it then declined, but remained above that of vehicle-treated controls during the 4-h time-course ($P<0.05$ at 150 and 180 min). The mean increase in VO_2 during the 4.0-h experiment for duloxetine-treated group was 0.52 ml/kg$^{0.75}$/min compared to an increase of 1.21/ml kg$^{0.75}$/min for metabolite 2-treated animals. Bupropion (30 mg/kg, p.o.) caused a rapid rise in VO_2, with a peak stimulation of 25% at 30 min posttreatment; VO_2

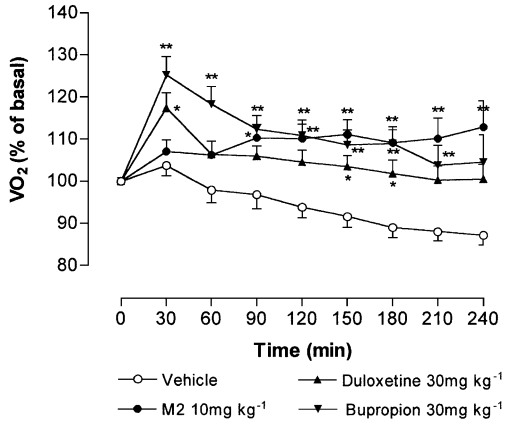

Fig. 6. Effect of metabolite 2 (10 mg/kg, p.o.), duloxetine (30 mg/kg, p.o.) and bupropion (30 mg/kg, p.o.) on oxygen consumption. Results are presented as percentage changes from basal values ($n=8$). * $P<0.05$, * * $P<0.01$ vs. vehicle control (Dunnett's test).

remained significantly higher than vehicle-treated controls until 210 min (Fig. 6).

4. Discussion

Studies in vitro using brain tissue preparations have shown that sibutramine is a weak reuptake inhibitor of noradrenaline in the rat and of 5-HT in the human, whereas metabolites 1 and 2 are potent reuptake inhibitors of both monoamines (Luscombe et al., 1989; Cheetham et al., 1993, 1996; Heal et al., 1998) These metabolites of sibutramine are approximately equal in potency to the selective noradrenaline reuptake inhibitor, desipramine and the selective 5-HT reuptake inhibitor, fluoxetine (Cheetham et al., 1993, 1996).

In terms of effects on food intake, results from the present investigation demonstrate that acute administration of metabolite 2, like its parent, sibutramine, evokes a marked decrease in food intake which lasts ≥ 24 h. It is not known whether the long-lasting effect of metabolite 2 on food intake is partly behaviorally related, as food consumption occurs largely during the dark phase (light-off period) and feeding behavior was not examined in the present study. However, there is some evidence to suggest that sibutramine, the parent compound, preserves the behavioral satiety sequence and advances the onset of resting (Halford et al., 1988 for review). Jackson et al. (1997b) have shown that the hypophagia induced by sibutramine could be mimicked by combined treatment with a selective noradrenaline reuptake inhibitor, nisoxetine, and a selective 5-HT reuptake inhibitor, fluoxetine, when individually these drugs had no effect on food intake, thus, demonstrating both noradrenaline and 5-HT mechanisms contribute to sibutramine-induced hypophagia. Consistent with this observation, sibutramine-induced hypophagia can be reversed by various adrenoceptor antagonists, i.e. prazosin (α_1), metoprolol (β_1) and 5-hydroxytryptaminergic, i.e. metergoline (nonselective), ritanserin ($5\text{-HT}_{2A/2C}$), SB 200646 ($5\text{-HT}_{2B/2C}$) receptors (Stricker-Krongrad et al., 1996; Jackson et al., 1997a). However, the inhibitory effects of the 5-HT receptor antagonists are generally less profound and sometimes less consistent than those of the noradrenergic antagonists, particularly prazosin. Thus, Grignaschi et al. (1999) observed no effect of metergoline (nonselective) or ritanserin ($5\text{-HT}_{2B/2C}$) on sibutramine-induced hypophagia in food-deprived rats, but they did find a partial reversal with SB 206553 {5-methyl-1-(3-pyridyl-carbamoyl)-1,2,3,5-tetrahydropyrrolo [2,3-f]indole; $5\text{-HT}_{2B/2C}$}, whilst in a parallel study to this one, metergoline was not observed to prevent the suppression of food intake by metabolite 2 on freely feeding rats (Liu and Stock, unpublished observations).

On the output side of the 'energy balance' equation, the results presented confirm the profound thermogenic action of metabolite 2. Thus, this compound evokes a sustained increase of VO_2 in rats at thermoneutral temperature (29 °C) as reported previously by Connoley et al. (1999) and metabolite 2 was also found to potentiate the colonic temperature of rats measured at room temperature (21 ± 1 °C). It should be noted that both colonic temperature (measured at 21 ± 1 °C) and oxygen consumption (measured at 29 °C) in vehicle-treated animals declined gradually during the course of experiments in the morning when the measurements were made. This decline is repeatedly observed and probably reflects a decrease in the diet-induced thermogenesis resulting from the last few nocturnal meals consumed just before the start of the light phase (07.00 h). By using a ganglionic blockade, chlorisondamine, we have shown in our previous study that the activation of thermogenesis by metabolite 2 (measured by VO_2) results from central stimulation of efferent sympathetic nerves to the specific effector, via brown adipose tissue (Connoley et al., 1999). Again, the effect of sibutramine and its active metabolites on VO_2 could be mimicked by coadministration of nisoxetine and fluoxetine, but neither drug given separately indicating reuptake inhibition of noradrenaline and 5-HT contributes to sibutramine's thermogenic effect (Connoley et al., 1999). This conclusion is supported by monoamine antagonist experiments in the investigation of Connoley et al. (1999) and by the more recent findings that the metabolite 2-induced increase in colonic temperature is inhibited by prazosin (α_1), high-dose propranolol (β_3 in addition to β_1 and β_2) and metergoline (5-HT, nonselective) (Liu and Stock, unpublished observations).

Duloxetine is also a noradrenaline and 5-HT reuptake inhibitor which has been shown to reduce food intake in food-deprived rats (Katoh et al., 1995) and in freely feeding rats during the dark phase (Jackson et al., 1997a). Results from the present study show that duloxetine reduces food intake of rats during the 8-h light period. Duloxetine's effect on food consumption is neither as potent, nor as long-lasting, as that of metabolite 2. Thus, the 30 mg/kg dose of duloxetine suppressed feeding to approximately the same degree as 10 mg/kg of metabolite 2. Similarly, duloxetine reduced cumulative food intake over 8-h consumption, but

not 24 h, whereas metabolite 2 profoundly suppressed feeding ≥24 h. The thermogenic property of duloxetine is evident as shown by the higher colonic temperature and oxygen consumption, although its effects were less profound than those of metabolite 2. Although sibutramine (predominantly via its active metabolites) and duloxetine are both noradrenaline and 5-HT reuptake inhibitors (Luscombe et al., 1989; Cheetham et al., 1993, 1996; Wong et al., 1993; Kasamo et al., 1996), sibutramine and metabolite 2 are more potent and efficacious in reducing food intake and increasing thermogenesis than duloxetine (Jackson et al., 1997b; this study). One explanation of these findings is enhanced noradrenergic function via α_1-adrenoceptors is a key driver of both effects and the reuptake inhibition profiles of metabolites 1 and 2, i.e. noradrenaline> 5-HT, are better suited to the induction of hypophagia and thermogenesis than that of duloxetine, i.e. 5-HT>noradrenaline. Although duloxetine was less potent than metabolite 2 in stimulating oxygen consumption (at 29 °C) and in increasing colonic temperature (at 21 °C), there was no difference in the requirement of exogenous heat in the cold (−8 °C) between the two compounds. The difference in thermogenic responses under different ambient temperature has been reported previously with the β-adrenoceptor agonist, isoprenaline (Carlisle and Stock, 1992). The increased barpress activity induced by metabolite 2 appeared to be a nonthermoregulatory behaviour, which was not observed after duloxetine treatment. The fact that both metabolite 2 and duloxetine caused a reduced colonic temperature after a 60-min test in the cold (−8 °C), suggests that both compounds may have decreased the "set point" of the thermostat, or a preferred body temperature due to the inability to activate thermogenesis. Such a phenomenon has been seen with other compounds which activate noradrenergic function, including noradrenaline, isoprenaline and ephedrine (Carlisle and Stock, 1999).

Bupropion is a monoamine reuptake inhibitor that is useful in the treatment of both depression and nicotine addiction (Ascher et al., 1995; Hughes, 2000). Its pharmacological profile is quite different from that of sibutramine or duloxetine because it is catecholamine-specific thereby lacking efficacy against 5-HT (Hyttel, 1982; Richelson and Pfenning, 1984). Bupropion's preferential action for dopamine versus noradrenaline in vitro (Hyttel, 1982; Richelson and Pfenning, 1984) is confirmed in vivo by the finding that this drug does not inhibit ptosis in rodents induced by monoamine depleting agents (Heal et al., 1992), unlike sibutramine, duloxetine and various other reuptake inhibitors either of noradrenaline plus 5-HT or noradrenaline alone (Buckett et al., 1988; Luscombe et al., 1989; Heal et al., 1992; Katoh et al., 1995). However, unlike sibutramine, bupropion enhances dopaminergic function in vivo, as shown by its ability to increase spontaneous locomotor activity, to evoke circling in unilaterally nigrostriatal-lesioned rats and to substitute for D-amphetamine in drug discrimination testing (Blitzer and Becker, 1985; Nielsen et al., 1986; Heal et al., 1992). The finding that subjects receiving bupropion gained less weight compared with those on placebo (Harto-Truax et al., 1983; Jorenby et al., 1999) led us to investigate the potential effect of this rather different monoamine reuptake inhibitor on energy balance (food intake and thermogenesis) also. Bupropion increased the colonic temperature of rats; a dose of 30 mg/kg, p.o. is approximately as efficacious as 10 mg/kg of metabolite 2 at stimulating thermogenesis (as determined by oxygen consumption and the requirement for exogenous heat at −8 °C). However, bupropion had no effect on food intake, suggesting that the weight-reducing effect of bupropion in the observed human studies (Harto-Truax et al., 1983; Jorenby et al., 1999) is probably due to its ability to activate thermogenesis. We are currently conducting further studies to elucidate the mechanism(s) of the thermogenic effect of bupropion.

Overall, this investigation into the actions of various monoamine reuptake inhibitors on food intake and energy expenditure has revealed that sibutramine's metabolite 2, with an in vivo profile of noradrenaline>5-HT>dopamine powerfully suppresses food intake and is profoundly thermogenic, whereas duloxetine (5-HT>noradrenaline) is much weaker in both respects. Bupropion (dopamine>noradrenaline) is profoundly thermogenic, but does not decrease food intake. If these results can be extended to man, they suggest that sibutramine probably reduces weight by decreasing calorie intake and increasing energy expenditure as suggested by various clinical studies (Rolls et al., 1998; Astrup et al., 1998; Hansen et al., 1998, 1999). Moreover, they also suggest that duloxetine is likely to exhibit similar, but weaker, antiobesity effects in man, whilst the reported weight-reducing effect of bupropion is likely to be due to the activation of thermogenesis.

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