Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 83 (2006) 517–527
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Locomotion is the major determinant of sibutramine-induced increase in energy expenditure

Valeria Golozoubova *, Frank Strauss, Kjell Malmlöf

Diabetes Pharmacology, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Måløv, Denmark

Accepted 7 March 2006
Available online 2 May 2006

Abstract

The anti-obesity effect of the serotonin and noradrenaline reuptake inhibitor sibutramine has been attributed to a dual mechanism involving a reduction of food intake and an increase in energy expenditure. This dual action increases the possibilities for induction of a negative energy balance, the principal goal of an anti-obesity treatment. To elucidate the mechanism behind sibutramine-induced increase in energy expenditure, we applied indirect calorimetry combined with monitoring locomotor activity and body temperature. We confirm that sibutramine has both anorectic and thermogenic effects. In addition, we show here that sibutramine also causes a dose-dependent increase in locomotor activity (LMA) of rats, occurring in parallel with increase in energy expenditure. The dose of sibutramine necessary to induce an effect on locomotion and energy expenditure was only marginally higher than the dose sufficient to induce a significant reduction of food intake. The relation between LMA and energy expenditure was similar to that found with D-amphetamine, which causes both hyper-locomotion and increased energy expenditure, but was different from 2,4-dinitrophenol which causes increase in energy expenditure but not in locomotion. The effect of sibutramine (20 mg/kg) on energy expenditure was not inhibited by the non-selective 5-HT receptor antagonist, metergoline (1 mg/kg), or a high dose (20 mg/kg) of the non-selective β-blocker propranolol, but was blocked by D1 dopamine receptor inhibitor SCH 23390 (0.3 mg/kg). Therefore, we conclude that the effect of sibutramine on energy expenditure in rats is predominantly due to a dopamine-dependent increase in locomotor activity.

Keywords: Sibutramine; Obesity; Thermogenesis; Locomotor activity; Food intake; Body temperature; Dopamine; Serotonin; Noradrenaline; Brown adipose tissue

1. Introduction

The serotonin (5-HT) and noradrenaline (NA) re-uptake inhibitor sibutramine, which is marketed in the United States as Meridia® and in many other countries as Reductil®, has a weight-reducing effect in rodents and humans (for review see (Finer, 2002; Luque and Rey, 2002; Nisoli and Carruba, 2000). Presently, sibutramine is one of the few compounds that are approved by FDA for chronic treatment of obesity.

The effect of the compound, and its two active metabolites, desmethylsibutramine (M1) and didesmethylsibutramine (M2), is thought to be due to a dual mechanism: a reduction of energy intake by increasing satiety/decreasing hunger, and an increase in energy expenditure (reviewed in e.g. (Luque and Rey, 2002)).

The sibutramine-dependent reduction of food intake is well established (Brown et al., 2001; Casado et al., 2003; Jackson et al., 1997). However, the thermogenic effect of sibutramine and its relative contribution to drug-induced weight loss has not been extensively examined. Although it has been demonstrated that sibutramine increase energy expenditure in rats in a dose-dependent manner (Connoley et al., 1999; Liu et al., 2002a) relatively little is known about the mechanisms underlying this effect.

An increase in energy expenditure can be due to an increase in obligatory/facultative thermogenesis, a direct effect. It can also be a consequence of an increase in physical activity, an indirect effect. The sibutramine-induced increase in energy expenditure

Abbreviations: LMA, locomotor activity; DNP, 2, 4-dinitrophenol; 5-HT, 5-hydroxytryptamine; NA, noradrenaline; SCH 23390, [*R*]-(+)-chloro-2,3,4,5-tetrahydro-5-phenyl-1 *H*-3benzazepin-al hemimaleate; M1, M1, secondary amine metabolite of sibutramine; M2, M2, primary amine metabolite of sibutramine.

* Corresponding author. Tel.: +45 44434394; fax: +45 44424486.
E-mail address: vgo@novonordisk.com (V. Golozoubova).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.03.014

may be due to either one or by both of these mechanisms, since both serotonin and noradrenaline systems are important regulators of both thermogenesis and locomotion.

In support of facultative thermogenesis as the reason of increased energy expenditure, Connoley et al. (1999) observed that the sibutramine-induced increase of energy expenditure was inhibited by a combination of high doses of β-adrenoreceptor antagonists. In addition, glucose utilization in brown adipose tissue was massively (18-fold) increased in rats exposed to sibutramine, indicating a high level of metabolic activity in this tissue. This suggests that sibutramine induces thermogenesis in brown adipose tissue by stimulation of noradrenaline release from sympathetic nerve terminals.

On the other hand, Glick et al. (2000) demonstrated that treatment with sibutramine or with either of its two metabolites results in a significant increase in locomotor activity (LMA) in rats, suggesting that heat production could be the result of a behavioural response. It was not established however, whether this increase in LMA represents only an acute response to the new environment, a "novelty stress", which is an inherent feature of this test, or if there is a sustained effect of the compound on locomotor activity.

The present study was performed to elucidate whether sibutramine-induced increase in locomotor activity contributes to the overall increase in energy expenditure caused by the compound.

2. Methods

2.1. Animals

Studies were conducted employing male Sprague–Dawley rats obtained from a local supplier (Moellegaard Breeding & Research Center A/S, Denmark). Animals were kept under 12/12 L/D cycle in temperature controlled rooms (21–22 °C). All experiments were conducted under ethical permit issued by the Danish Animal Experiments Inspectorate and followed the ethical guidelines established by Novo Nordisk A/S. Ethical standards are consistent with Council Directive 86/609/EEC of The Council of the European Communities and European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

2.2. Compounds

Sibutramine hydrochloride was custom-synthesised by Chem Pacific, USA (batch No. CHP 1101011), and its purity was checked by HPLC. 2, 4-dinitrophenol (DNP), noradrenaline bitartrate (Arterenol) D, L-propranolol and metergoline were from Sigma (Sigma-Aldrich Denmark, Copenhagen, Denmark); D-amphetamine and SCH 23390 were from Sigma-RBI (Sigma-Aldrich Denmark, Copenhagen, Denmark). All compounds that were administrated orally (sibutramine, DNP, D-amphetamine, metergoline) were dissolved in distilled water. All compounds administrated intraperitoneally (noradrenaline, propranolol) or subcutaneously (SCH 23390) were dissolved in sterile 0.9% saline.

2.3. Locomotor activity (LMA) in a novel environment.

The plastic activity cages (28 × 28 × 35 cm, open on top) were equipped with two movement detection frames (Ellegaard Systems A/S, Faaborg, Denmark). The first frame was placed at a height of ca. 1 cm, detecting movements in the "*X–Y*" axis for recordings of locomotion; the second frame was placed at the height of ca. 12 cm, detecting "vertical activity" for recordings of rearing. Four infrared-activated photo-cell senders/receivers placed on the side of the cage were located on each of the two frames, and the system was connected to an electronic event recorder. Motility (*X–Y* axis) and rearing were counted when photo-beam interruptions were longer than 50 ms. To avoid effects of external factors, the cages were placed in closed ventilated containers, equipped with a light source (measured light intensity 2.6 lux). About 2 h before the start of each experiment, rats were transferred to the laboratory and different doses of sibutramine were administrated orally, 1 h before commencement of measurements. Recordings were started immediately after the rats had been placed into the activity chambers. Photo-beam interruptions were recorded for the entire 30 min session, comprising three 10-min intervals (0–10, 10–20, and 20–30 min). For some of the doses, the observation time was extended for up to 4 h.

2.4. Energy expenditure (EE)

Energy expenditure (oxygen consumption) was measured by indirect calorimetry (Oxymax equal flow system, Columbus Instruments, Columbus, U.S.A.). The system was calibrated daily, before the start of measurements. The temperature in the chambers was 22–22.5 °C. Oxygen consumption was calculated per metabolic weight (live weight in $kg^{0.75}$).

Animals were weighed and placed into metabolic chambers with constant ventilation (flow 1.8 l/min). In the chambers, rats had free access only to water. After an adaptation period of 2.5 h, animals received vehicle or sibutramine, and measurements continued for 3 h. After this, animals were returned to their home-cages. Antagonists and vehicle were administrated after 2.5 h adaptation, 30 min (unless otherwise indicated) prior to sibutramine/water and measurement continued for 4–6 h post-dosing.

For parallel measurements of oxygen consumption and activity, the Oxymax system was used in combination with either photo-cell locomotor activity recording system (Automatik Partner APS, Kastrup, Denmark), or, in experiments performed with rats in which telemetry senders had been implanted, with telemetry system (DSI, St. Paul, MN U.S.A.).

In the photo-cell system, three senders/receivers were placed on the long side of each Oxymax chamber, and one on the short. The frame carrying the photo cells was placed at a height of about 3 cm above the floor of the chamber. Oxymax data, and cumulative count of photobeam interruptions (*X–Y* axis only) were collected for each 14.5-min interval.

When the telemetry system (DSI, St. Paul, MN U.S.A.) was used, data of locomotor activity and core temperature were collected every 1 min or every 2.4 min. For locomotor activity,

average locomotion was further recalculated per interval corresponding to oxygen consumption measurements. Parallel measurements of oxygen consumption were performed with a sampling interval of 9 or 12 min. Senders, suitable for recording of locomotor activity and core body temperature in rats (type TA-F40, DSI, St. Paul, MN U.S.A.) were tested and implanted into the abdominal cavity under isofluran anaesthesia at least two weeks before the measurements. Immediately before the operation, and for 3 days after the operation, rats also received nonsteroidal anti-inflammatory drug rimadyl (caprofen, Pfizer ApS, Ballerup, Denmark) in a dose of 5 mg/kg. Once implanted, the senders were turned on and allowed to stabilize for at least 24 h before the start of the recording. Occasional values of higher than 20 counts/min (for activity), and occasional values above 40 °C and below 35 °C (for the temperature) were regarded as electrical noise and were filtered out.

2.5. Food intake

The acute effect of sibutramine on food intake was measured in schedule-fed rats. Rats were placed two and two per cage with a plastic separator in between, so that rats had physical contact with each other, but were not able to reach each other's food. Rats were trained to consume food between 9.00 and 14.30 h (food available ad lib during this time interval but not at other times) for 2 weeks before the start of experiment. During this training period, rats were accustomed to handling and dosing. One day before the experiment, rats were weighed and allocated to weight-matched groups ($n=10$). During the experiment, vehicle (water) or sibutramine (3, 10 and 20 mg/kg) was given orally at 8.00 h. Pre-weighed food was given at 9.00 h, and was collected and weighed again at 14.30 h.

2.6. Chronic effect of sibutramine

Chronic effects of sibutramine were measured in ad libitum fed rats. Rats, placed two and two per cage with a plastic separator in between (as described in Section 2.5), were divided in 2 weight-matched groups, and received sibutramine or water once daily, 1.5 h before lights were turned off. Food intake and body weight were measured daily. After 11 days of treatment, 4 rats from each group were placed into metabolic chambers, and received sibutramine after 2.5 h of adaptation. Effects on energy expenditure and locomotor activity were followed for 3 h post-dosing with a sampling interval of 21.75 min.

2.7. Statistical analysis

Unless otherwise indicated, one-way ANOVA followed by Dunnett's multiple comparison test was used.

3. Results

3.1. Effect of sibutramine on spontaneous locomotor activity in a novel environment

Sibutramine elicited a pronounced effect on LMA in rats exposed to novel environment in activity cages (Fig. 1). Both locomotion count (Fig. 1A) and rearing (Fig. 1B) were increased.

Within the first 10 min in the chamber, the period during which the reaction to the "novelty" of the environment was the strongest, LMA of sibutramine-treated rats did not differ much from control animals (Fig. 1). During the two following time intervals, 10–20 and 20–30 min, exploratory activity of control rats decreased and, consequently, their LMA counts were lower than during the initial 10 min in the chambers. In contrast to control rats, rats given sibutramine remained active during these time intervals, and their LMA counts were consequently much higher compared with control rats. This effect was clearly dose-dependent. The activity of rats, treated with 3 mg/kg, did not differ from control rats at any time-point, whereas the rats that received 10, 20 or 30 mg/kg, were increasingly active (Fig. 1). Additional test with 3 doses, 3, 10 and 20 mg/kg showed that this increase in locomotor activity in response to the higher doses of sibutramine was sustained, and the difference between groups was statistically significant after at least 4 h, while a dose of 3 mg/kg had no effect at any time (not shown). Taken together, these data indicate that although sibutramine treatment does not enhance the immediate reaction to novel environment, treatment with the compound results in a sustained increase of locomotor activity.

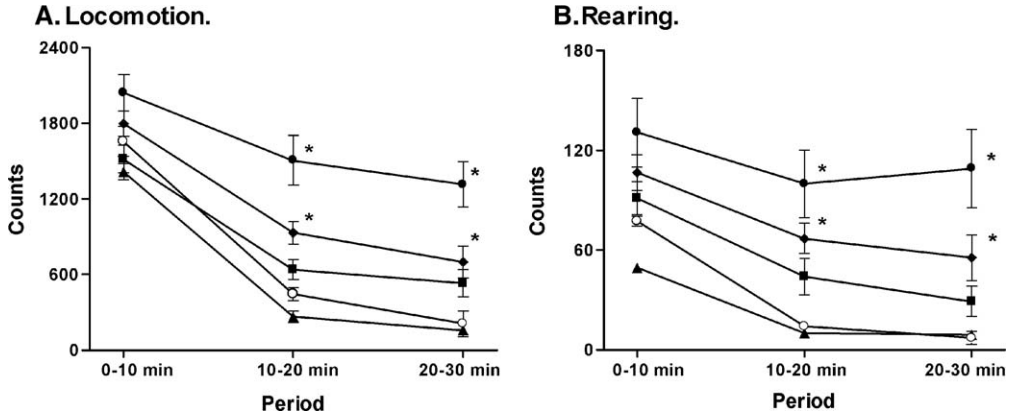

Fig. 1. Locomotor activity (A) and rearing (B) in a novel environment. Rats ($n=8$ per group) received vehicle (empty circles) or sibutramine (filled symbols: 3 mg/kg — triangles, 10 mg/kg — squares, 20 mg/kg — rhombs, 30 mg/kg — circles) 1 h before they were placed into the novel environment activity chambers (see Methods for details). Data were collected over three 10 min periods. * $p<0.05$, one-way-ANOVA followed by Dunnett's multiple comparison test.

3.2. Effects of sibutramine on locomotor activity, energy expenditure and body temperature in rats habituated to metabolic chambers

To address the questions of whether the effect of sibutramine on locomotor activity was sustained also in the animals habituated to the environment, rats were allowed to pre-adapt to the metabolic chambers (Oxymax system) for 2.5 h before receiving vehicle or sibutramine. Also, in this setting, where the "novelty factor" was reduced to a minimum, a clear increase in locomotor activity was observed (Fig. 2A). The effect appeared ca. 30 min after compound administration, and was dose-dependent. No effect was seen with 3 mg/kg, some increase was observed with 10 mg/kg, and massive effects were seen with 20 and 30 mg/kg. When calculated as total activity during 3 h after compound administration, the effect of 20 and 30 mg/kg was statistically significant. About 3–4 h post-dosing, some of the rats receiving 20 mg/kg, and all the rats receiving 30 mg/kg showed signs of anxiety and behavioural abnormalities. They were constantly moving, overreacted to touch, some of the rats treated with 30 mg/kg even showed signs of stereotypic behaviour. The dose 30 mg/kg was therefore omitted from further tests.

Sibutramine induced a clear dose-dependent increase in energy expenditure (Fig. 2B). As with LMA, no effect was seen in rats receiving 3 mg/kg, some increase was observed with 10 mg/kg, and a significant effect (calculated as area under the curve for the 3 h post-dosing period) was seen in rats receiving 20 or 30 mg/kg. Similar to locomotor activity, the response started to be pronounced ca. 30 min after compound administration. Thus, the time-schedule and dose-dependence of the sibutramine effect on LMA and energy expenditure were virtually the same.

Rectal temperature was measured before administration of sibutramine, and at the end-point of experiment, 3 h after dosing. As expected, an increased energy expenditure and locomotor activity were accompanied by a dose-dependent increase in rectal temperature (not shown).

3.3. Comparison of the effects of sibutramine, DNP and amphetamine

Using a combination of Oxymax system and telemetry (DSI Instruments), the magnitude of the response to sibutramine on energy expenditure, LMA and core body temperature was studied in more detail. The effects were compared with the effects of 2,4-dinitrophenol (DNP), a compound known to increase mitochondrial thermogenesis (reviewed in Harper et al., 2001), but not locomotor activity. Effects of D-amphetamine, a compound well known to induce massive locomotor activation (reviewed in Antoniou et al., 1998) were also compared.

Rats, equipped with telemetry-sensors were allowed to settle in the chambers for 2.5 h before vehicle, sibutramine (20 mg/kg p.o.), DNP (20 mg/kg p.o.) or amphetamine (2 mg/kg p.o.) was administered. According to our experience this dose of amphetamine is sufficient to induce hyper-locomotion, but is not high enough to induce stereotypic behaviour.

As expected, dosing per se caused a transient increase in all of the three parameters measured. However, while the "handling effect" had disappeared already after about 30–60 min, specific effects of the compounds were clearly visible at later time-points (Fig. 3). All three compounds caused a clear, statistically significant increase in energy expenditure ($p<0.05$ for amphetamine and $p<0.01$ for DNP and sibutramine; Fig. 3A) and core body temperature ($p<0.01$; Fig. 3C). A treatment-specific increase in locomotor activity was clearly pronounced for D-amphetamine and sibutramine ($p<0.01$), but was statistically insignificant for DNP (Fig. 3B). The small increase in activity seen in DNP group was primarily associated with drinking.

The maximum effect on energy expenditure of DNP and amphetamine was comparable, although effect of DNP was significantly extended. The effect of sibutramine was somewhat lower, and the response was sustained for a longer time-period.

DNP and D-amphetamine produced an increase in core body temperature by 0.7–0.8 °C. This increase was well pronounced already at 60 min following dosing. The equivalent increase in

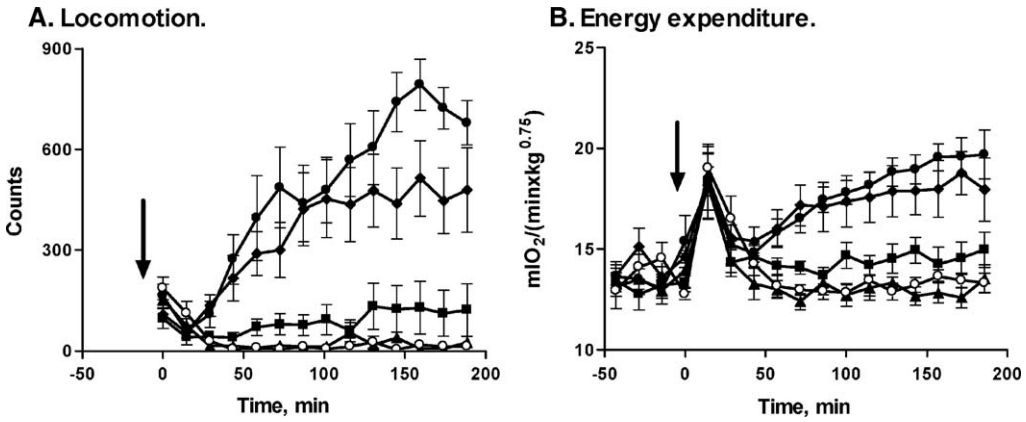

Fig. 2. Parallel measurements of locomotor activity (A) and energy expenditure (B) in rats habituated to the chambers (Oxymax system). Rats ($n=6$ per group) received vehicle (empty circles) or sibutramine (filled symbols: 3 mg/kg — triangles, 10 mg/kg — squares, 20 mg/kg — rhombs, 30 mg/kg — circles) at the time-point indicated by the arrow. Recording of energy expenditure was started at the time-point animals were placed in the chambers (only last 40 min of 2.5 h adaptation period is shown); recording of activity was initiated at the time-point of injection.

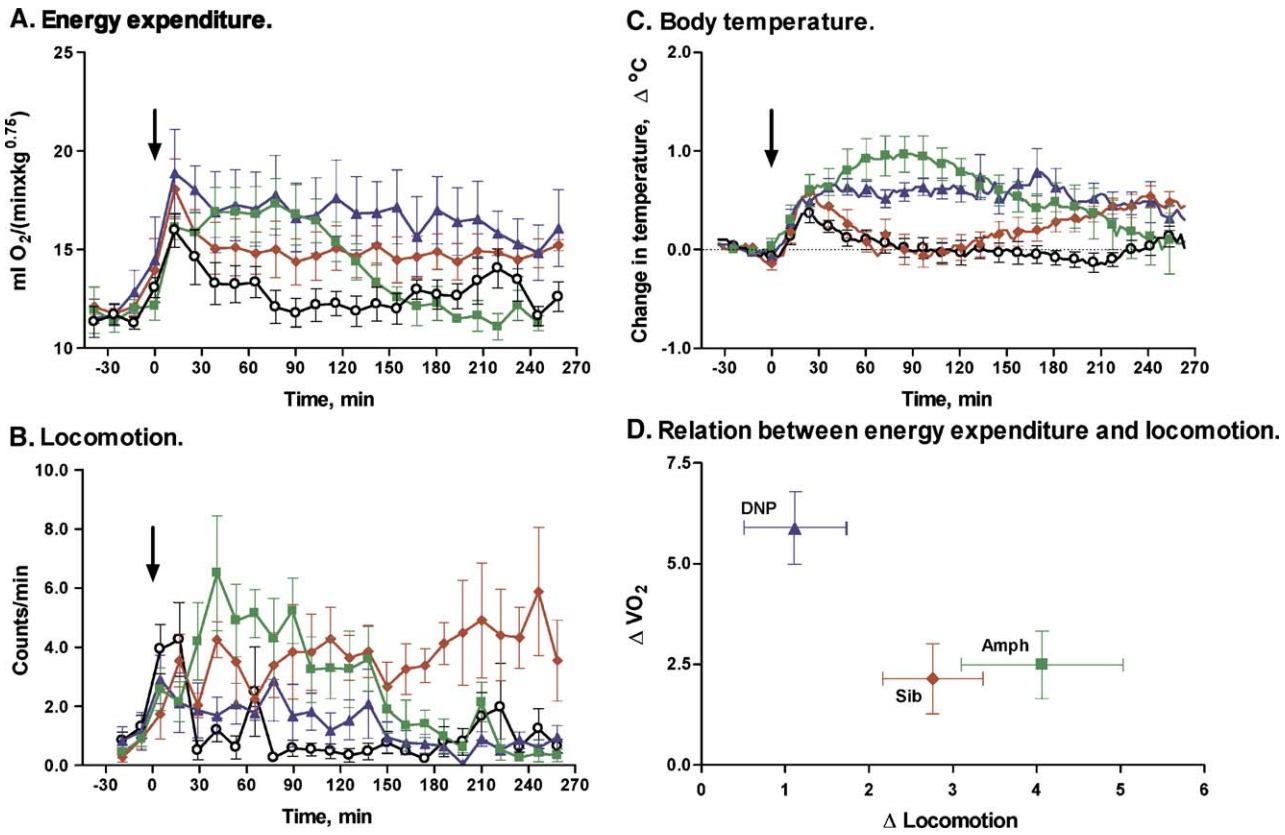

Fig. 3. Effects of DNP, sibutramine and D-amphetamine on energy expenditure (A), locomotor activity (B) and body temperature (C). Rats, carrying telemetry senders (n=5–6 per group), received (p.o.) vehicle (black empty circles), DNP (20 mg/kg, blue triangles), sibutramine (Sib, 20 mg/kg, red rhombs) or D-amphetamine (Amph, 2 mg/kg, green squares) at the time-point indicated by arrow. Recordings were done for 2 h before and 4.5 h after compounds were administrated. Data were collected every 12 min for energy expenditure; every 2.4 min and recalculated as average over 12 min interval for locomotor activity, and every 2.4 min (only every 5th data point shown as symbol±SE) for body temperature. In D, average increase in energy expenditure above vehicle level plotted against the increase in locomotor activity during the corresponding time-period is shown. These data were calculated from results shown in A–B for the period between 25–168 min post-dosing.

core temperature in response to sibutramine, reaching 0.5 °C, was observed later and became pronounced after 2.5 h post-dosing (Fig. 3C).

The method for locomotor activity measurements performed in the present study was not sufficient to make a precise quantification of calorie expenditure per unit of activity. For that reason, we attempted to correlate average locomotor activity and energy expenditure of rats during the period of treatment-specific increase in oxygen consumption. As seen in Fig. 3D, locomotor activity of D-amphetamine and sibutramine treated rats was much higher than the activity of either control or DNP-treated rats during the period of increased energy expenditure.

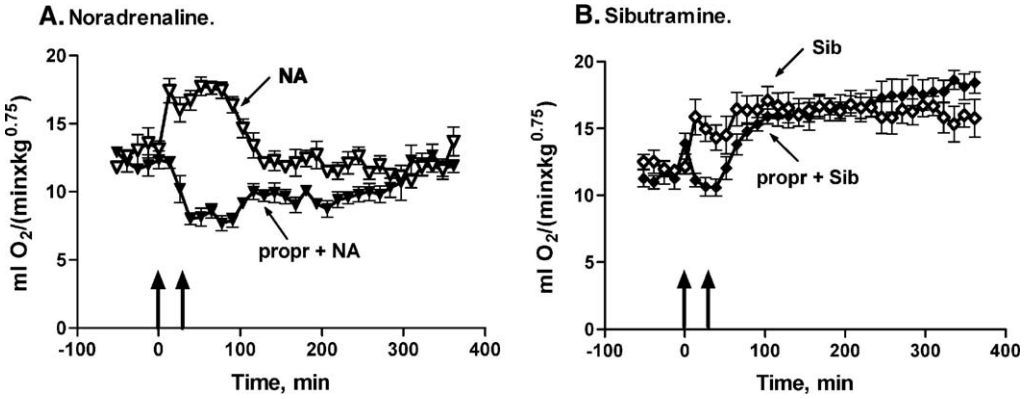

Fig. 4. Effect of high dose of propranolol on noradrenaline- (A) or sibutramine- (B) induced increase in energy expenditure. Rats (4–6 per group) were pre-treated with either saline (empty symbols) or with propranolol (propr, 20 mg/kg i.p., filled symbols); ca. 25 min later, rats received either noradrenaline (NA, 2 mg/kg i.p., A) or sibutramine (Sib, 20 mg/kg p.o., B), and recording continued for ca. 6 h, data being collected every 12 min. Arrows indicate the time-points of compounds administration.

Both the sibutramine- and D-amphetamine-induced increase in energy expenditure coincided with the increase in locomotor activity.

3.4. The contribution of the β-adrenergic system to sibutramine-induced increase in energy expenditure

In order to estimate the contribution of β-adrenergic receptors in the thermogenic response to sibutramine, we treated rats with either saline or with propranolol prior to sibutramine administration. A high dose (20 mg/kg i.p.) of propanolol was used in order to inhibit both β1/β2- and β3-adrenergic receptors.

The only effect of propranolol in itself was to reduce the magnitude of the acute "stress–response" to the injection (Fig. 4). At any later time-point, the combination of propranolol and water was indistinguishable from the combination of saline and water (not shown). As expected, β-adrenergically mediated thermogenic effect of the positive control, noradrenaline, was completely abolished by propranolol pre-treatment (Fig. 4A). Propranolol inhibited both the LMA-dependent and the presumed LMA-independent (brown adipose tissue-derived) components of the response to noradrenaline in rats kept at an ambient temperature of 20–22 °C. However, pre-treatment with propranolol did not prevent either sibutramine-induced increase in energy expenditure (Fig. 4B) or sibutramine-induced increase in LMA (data not shown).

The lack of the effect of propranolol on sibutramine-induced increase in energy expenditure was not due to insufficient amount of propranolol available in the system at the time-point of the response initiation (starts ca. 30 min post-dosing): single administration of 20 mg/kg propranolol was sufficient to completely inhibit effect of noradrenaline, independently on whether noradrenaline was given 25 min (Fig. 4A) or 1.3 h after propranolol (data not shown).

Thus, inhibition of β-adrenergic receptors did not block the effect of sibutramine on either LMA or energy expenditure.

3.5. The contribution of the serotonin system to sibutramine-induced increase in energy expenditure

To evaluate the contribution of serotonin system, rats were treated with non-selective 5-hydroxytryptamine receptor antagonist, metergoline (1 mg/kg) prior to administration of sibutramine. In itself, metergoline had no effect on energy expenditure (Fig. 5A) and had a minor effect on body temperature (Fig. 5B). Pre-treatment with metergoline did not prevent the sibutramine-induced increase in energy expenditure (Fig. 5A). However, metergoline completely abolished the sibutramine-induced increase in body temperature (Fig. 5B).

Thus, inhibition of 5-HT receptors did not block the effect of sibutramine on energy expenditure but had an effect on body temperature.

3.6. The contribution of dopamine-dependent locomotor activity to the sibutramine-induced increase in energy expenditure

Stimulation of locomotion is a well established effect of dopamine ((Missale et al., 1998) and references therein). In control rats, treatment with SCH 23390, an antagonist for D1-like family of dopamine receptors, did not cause any major reduction in either locomotor activity or energy expenditure. Pre-treatment with SCH 23390, however, completely blocked sibutramine-induced increase in both locomotor activity and energy expenditure (Fig. 6).

3.7. Effect of sibutramine on energy expenditure and food intake

The dose of sibutramine necessary to induce an increase in energy expenditure/locomotor activity (10 mg/kg, (Connoley et al., 1999) and the present study (Fig. 2)) was relatively high. In order to dissociate anorectic and locomotion-stimulating

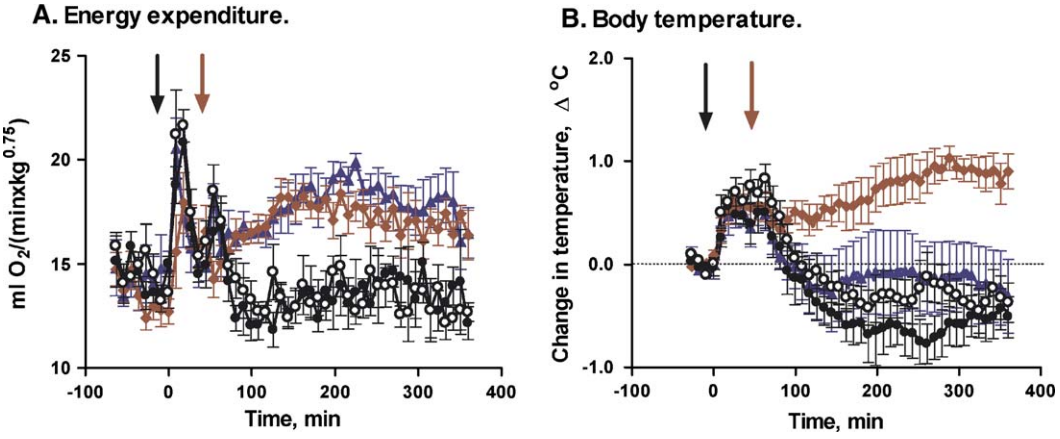

Fig. 5. Effect of metergoline on sibutramine-induced increase in energy expenditure (A) and body temperature (B). Rats (n=4–5 per group) were pre-treated with metergoline (1 mg/kg p.o.) or water; 45 min later, rats received sibutramine (20 mg/kg p.o.) or water. Combinations are shown: Water/Water in black (empty circles), Metergoline/Water in black (filled circles), Water/Sibutramine in red (rhombs) and Metergoline/Sibutramine in blue (triangles). Black arrows indicate time-points of administration of water/metergoline; red arrows indicate time-points of water/sibutramine administration. Sib — sibutramine; met — metergoline. Recordings continued for 6 h post-dosing, with data collection every 9 min for energy expenditure and every minute (only every 9th point is shown as symbol±SE) for body temperature.

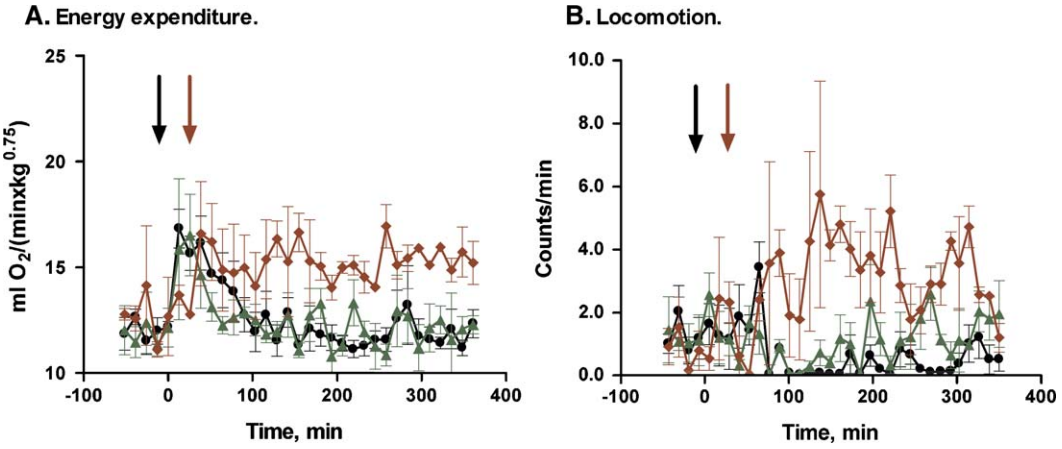

Fig. 6. Effect of SCH 23390 on sibutramine-induced increase in energy expenditure (A) and locomotor activity (B). Rats ($n=4$ per group) were pre-treated with SCH 23390 (0.3 mg/kg s.c.) and 25 min later received sibutramine (20 mg/kg p.o., green triangles) or water (p.o., black circles). Positive control (response to sibutramine in rats pre-treated with saline (s.c.) instead of SCH 23390) is shown in red. Black arrows indicate time-points of administration of water/SCH 23390; red arrows indicate time-points of sibutramine administration. Recording continued for 6 h post-dosing with data collection every 12 min.

effects of sibutramine, the effects on food intake were measured in schedule-fed rats with the same doses of sibutramine that were used in the energy expenditure study.

A low dose of sibutramine which was without effect on locomotor activity or energy expenditure (Fig. 2), did not cause any significant reduction of food intake (Fig. 7A). A dose of 10 mg/kg had a clear effect ($p<0.001$), 24±5% reduction of food intake and an even higher dose, 20 mg/kg resulted in a massive 58±5% ($p<0.001$) reduction of food intake.

The dose of 3 mg/kg did not become more effective with time: even if dosing continued for 3 days, the effect of this dose was still not very impressive (Fig. 7B) and did not result in any change of body weight (not shown).

We conclude that the window between the dose of sibutramine necessary to significantly reduce appetite and the dose sufficient to cause an increase of locomotor activity and energy expenditure is narrow.

3.8. Chronic effect of sibutramine

To establish whether the effect of sibutramine on locomotion/energy expenditure may contribute to long-term effect of sibutramine, rats were treated with sibutramine (10 mg/kg) for 11 days. Thereafter, the effect of the compound on locomotion and energy expenditure was measured. Rather unexpectedly, the effect of this dose of sibutramine on food intake was limited to the initial 5 days of treatment (Fig. 8C); thereafter food intake of sibutramine-treated rats was indistinguishable from control rats (Fig. 8C). Minor effect on locomotion (Fig. 8A) and energy expenditure (Fig. 8B) was still present after 11 days of treatment; the magnitude of this effect was similar to the effect seen after acute treatment (Fig. 2). These results suggest that the mechanisms behind the effects of sibutramine on food intake and energy expenditure/locomotion differ.

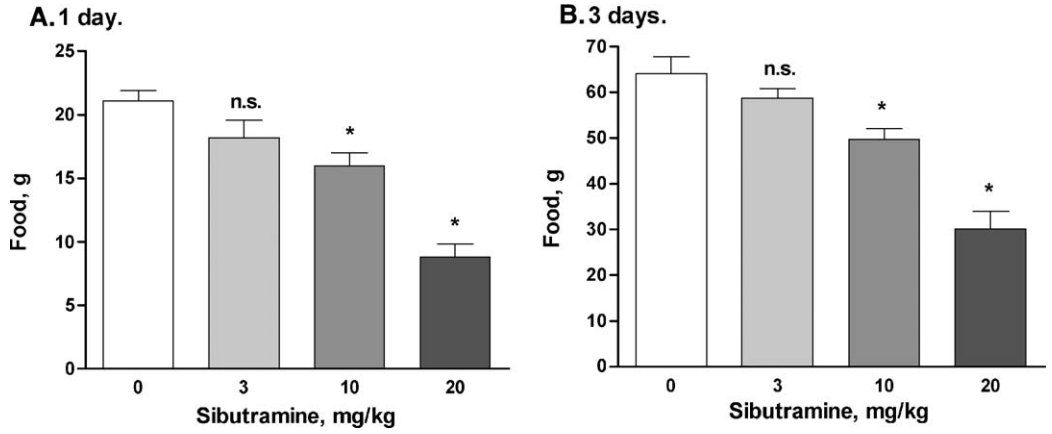

Fig. 7. Effect of sibutramine on food intake in schedule-fed rats: A — acute food intake; B — total food intake over three days of treatment. Rats ($n=10$ per group), trained to schedule-feeding (see Methods for details) received the indicated doses of sibutramine p.o. 1 h before the start of the feeding session. *$p<0.001$; n.s. — $p>0.05$.

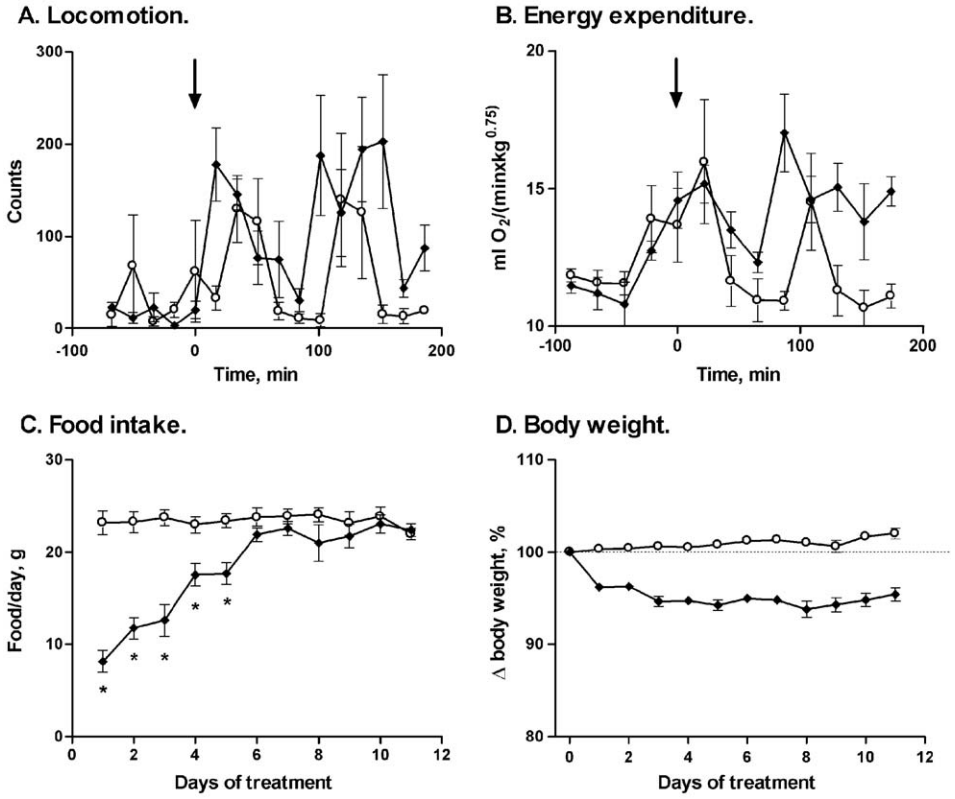

Fig. 8. Chronic treatment with sibutramine. Rats ($n=10$ per group) received water or 10 mg/kg of sibutramine daily for 11 days. On day 12, 4 rats from each group were placed to metabolic chambers 2.5 h prior to sibutramine dosing, and measurement of energy expenditure and locomotion continued for 3 h post-dosing. Locomotor activity and energy expenditure before and after compound administration on day 12 are shown in A and B, respectively. Effect in the post-dosing period for both parameters was significant ($p<0.05$, unpaired t-test). Daily food intake (*$p<0.01$) and change in body weight ($p<0.01$ on all treatment days, unpaired t-test) of these rats over the whole treatment period is shown in C and D, respectively. In some points, SEM is smaller than the symbols.

Body weight was clearly reduced during the initial period (effect on food intake and on energy expenditure); thereafter it was maintained on the same level until the last day of treatment (effect on energy expenditure but not on food intake, Fig. 8D). Therefore, the effect of this dose of sibutramine on locomotion/energy expenditure alone does not seem to be sufficient for continuation of weight loss, as rats remain weight-stable after food intake returns to the control level.

4. Discussion

In the present study, we have shown that sibutramine causes a sustained increase in locomotor activity, energy expenditure and body temperature. These effects were observed following doses of sibutramine that were in the same range as those necessary to induce an effect on food intake. The effects on locomotor activity and energy expenditure were inhibited by pre-treatment with the dopamine receptor antagonist, SCH 23390, but not by the non-selective inhibitor of 5-HT receptors metergoline or by a high dose of the non-selective β-adrenergic receptor antagonist propranolol. In view of this, the most probable explanation as to why sibutramine increases energy expenditure in rats is a dopamine-dependent increase in locomotor activity.

The results of the present study emphasize the principal importance of locomotor activity measurements in energy expenditure studies. The question of how the increase in energy expenditure is brought about becomes of particular value in characterization of the new anti-obesity drug candidates. Direct correlation of locomotor activity and energy expenditure at the corresponding time-points is a challenging task from the technological point of view, still waiting for a satisfactory resolution. Indeed, both methods of LMA evaluation used in our study allowed us to conclude that there was an increase in locomotor activity, and to identify the dose of the compound necessary to induce such an increase. However, neither of these methods in their present form allows to quantitatively correlate energy expenditure ("true" units) and locomotor activity (arbitrary units, all movements are registered, but no evaluation of energy requirements for different types of movements can be done).

The unexpected observation that the effect of sibutramine on locomotor activity, and, consequently on energy expenditure, was dependent not (or not only) on 5-HT or noradrenaline, reuptake of which sibutramine is the inhibitor of, but on dopamine, is, in fact in agreement with literature data. Several authors have shown that sibutramine and its metabolites are reuptake inhibitors not only of serotonin and noradrenaline, but also of dopamine (Rowley et al., 2000; Balcioglu and Wurtman, 2000; Glick et al., 2000). However, relatively high doses of sibutramine were necessary to induce an increase in extra neuronal dopamine levels (6 mg/kg i.p., (Rowley et al., 2000) or to enhance the net flux of dopamine from rat striatal

and hypothalamic neurons in vivo (5 or 10 mg/kg i.p. but not at 2 mg/kg i.p., (Balcioglu and Wurtman, 2000).

Although the dopamine system was clearly important for stimulation of locomotor activity, and hyper-locomotion is a well established dopamine-dependent behaviour, other systems may also be involved. The interplay of adrenergic, 5-HT and dopamine systems in regulation of sibutramine-induced increase in energy expenditure, locomotor activity and body temperature needs further elucidation. The fact that bupropion, a weak monoamine reuptake inhibitor selective for dopamine and noradrenaline, but inactive against 5-HT, has an effect on energy expenditure and colonic temperature in rats comparable to the effect of M2, the major pharmacologically active metabolite of sibutramine (Liu et al., 2002a), shows the importance of adrenergic and dopaminergic systems. On the other hand, Liu et al. (2002b) has shown that non-selective 5-HT receptor antagonist, metergoline, partially prevented M2-induced increase in body temperature, which suggests a contribution of the 5-HT system as well. In the present study, we also observed inhibition of sibutramine-induced increase in body temperature by metergoline. However, metergoline pre-treatment did not prevent the sibutramine-induced increase of energy expenditure. Thus, there is a clear distinction between effects of this inhibitor on body temperature and on energy expenditure. One possible explanation of this could be that metergoline causes a general reduction of body temperature by e.g. increased vasodilatation, without stimulating a compensatory increase in energy expenditure. Indeed, metergoline in itself had a body temperature-reducing effect, marginal in our study (core body temperature measured), much more substantial in the study of Liu et al. (colonic temperature measured, (Liu et al., 2002b)).

The β-adrenergic system does not seem to be involved in mediation of acute effect of sibutramine on energy expenditure and locomotor activity since neither of these effects were blocked by a high dose of non-selective β-blocker propranolol, which at the dose used acts as an inhibitor of β1, β2 and β3 adrenergic receptors. Indeed, the same dose of propranolol completely abolished the thermogenic effect of our positive control compound, noradrenaline, independent on whether noradrenaline was given 20 min or 1.3 h after propranolol. The lack of inhibition of sibutramine-induced thermogenesis cannot therefore be explained by either inactivity of propranolol, or by its short period of action. Although our results are somewhat unexpected in view of the original report by Connoley et al. (1999), in which β3-adrenergically mediated, brown adipose tissue-derived thermogenesis was suggested to be the main source of the thermogenic effect of sibutramine, our conclusion is supported by a later publication from the same group (Liu et al., 2002b). In that study, it was shown that a high dose of propranolol only to a minor extent could counteract M2-induced colonic temperature increase. A possible explanation for the discrepancy in the results is pharmacological: in the study of Connoley et al. (1999), not propranolol, but a combination of high doses of β1-selective (atenolol, 20 mg/kg) and β2-selective (ICI 118551, 20 mg/kg) antagonists was used. The selectivity of this combination, given at such a high dose, is low, and may therefore affect other receptors, possibly α2-adrenoreceptors, involved in regulation of brain monoaminergic function by inhibiting neuronal firing and release of noradrenaline, 5-HT and dopamine (Lahdesmaki et al., 2003).

The pharmacological profile of the effect of sibutramine on energy expenditure (dopamine, but not noradrenaline and not 5-HT) allows some predictions on the mechanisms of heat production. One important conclusion is that acute thermogenic response to sibutramine treatment does not originate in brown adipose tissue. Indeed, inhibition of β-adrenergic receptors, the main regulators of brown adipose tissue-derived thermogenesis, did not prevent or even reduce thermogenic response to sibutramine. In addition, there was no difference in the magnitude of the thermogenic response measured at thermoneutral temperature (Connoley et al., 1999) and at room temperature (present study). This would not be expected for a brown adipose tissue-derived thermogenesis. In animals, acclimatized to the temperature below thermoneutral, the effect should be smaller when measured at the acclimation temperature, than measured at thermoneutral temperature where thermogenic capacity of the tissue is already partially or completely utilized.

On the other hand, the observation that dose-dependence, time of action and response to inhibitors are identical for effects on locomotor activity and energy expenditure allows us to suggest that a significant part of the increase in energy expenditure is the consequence of increased locomotion.

Although our conclusion that locomotor activity, and not brown adipose tissue-derived thermogenesis, is the main determinant of sibutramine-induced acute increase in energy expenditure may be somewhat unexpected, our data do not contradict the literature. Glick et al. (2000) have shown that locomotor activity of naïve rats was increased after sibutramine treatment; also in this study, the difference between the dose causing a significant reduction of food intake (5 mg/kg i.p.), and the dose stimulating locomotor activity (10 mg/kg i.p.) was minimal.

An ability to increase energy expenditure is usually regarded as a beneficial quality for the appetite-reducing drugs. In a number of studies (Keesey and Corbett, 1990; Lazzer et al., 2004; Stock, 1989; Zwiauer et al., 1992), it was demonstrated that reduction of food intake/body weight is often associated with a reduction in energy expenditure as well, thus reducing the efficiency of weight-loss attempts. However, benefits of an increase in energy expenditure by means of restlessness is questionable, and should perhaps be regarded as a side-effect. In this respect, it would be desired that anorectic and locomotor effects of sibutramine could be separated. The dose of sibutramine necessary for acute inhibition of food intake in the present study (10 mg/kg) is higher than the doses (1–5 mg/kg) reported to be sufficient to cause a significant weight loss in obese mice and rats (Day and Bailey, 1998; Levin and Dunn-Meynell, 2000; Nakagawa et al., 2000; Stricker-Krongrad et al., 1995). However, it was found, that older rats with higher fat content were more sensitive to sibutramine than were younger rats with a lower carcass fat content (Strack et al., 2002), and higher dose of sibutramine is necessary to induce a significant reduction of food intake in lean, healthy animals compared to genetically obese or diet-induced obese rats (Stricker-Krongrad et al., 1995). In lean, growing animals, ED_{50} for the inhibition of

24 h food intake was reported to be approximately 5–8 mg/kg (Stock, 1997). In addition, we show that in contrast to obese animals, sibutramine-induced reduction of food intake in lean animals is sustained for only 5 days. It is important to note that sibutramine still has an effect on food intake at later time-points: the termination of treatment with the compound results in overeating and fast return to the initial body weight. The existence of such difference between lean and obese animals stresses the importance of the use of the same type of animals (either lean or obese) both in efficacy studies and for side-effect profiling of the new anti-obesity drug candidates.

Unlike the effect on food intake, the effect of sibutramine on locomotion/energy expenditure was sustained and possibly enhanced due to increased thermogenic capacity of brown adipose tissue upon chronic treatment (Giordano et al., 2002) after 11 days of treatment. At this point in time, food intake of rats treated with sibutramine was the same as food intake of control rats. Since body weight was also stable by then, the only possible contribution of somewhat increased energy expenditure to the overall energy balance would be prevention of the reduction of energy expenditure typical for starved animals.

Clinical data are not sufficient to allow a conclusion on whether a sibutramine-induced increase of energy expenditure as observed in the present study exists in humans, and if it does, whether this is associated with "restlessness". Indeed, reports exist that sibutramine induces an increase in energy expenditure (Hansen et al., 1998), has no effect on energy expenditure (Seagle et al., 1998; Starling et al., 2001), or that it prevents weight-loss associated decline in energy expenditure (Hansen et al., 1999). More data is needed to clarify this issue.

In the present study, we compared sibutramine to the compound that induce high level of energy expenditure without or almost without increase in locomotor activity (e.g. DNP), and compounds that cause an increase in both energy expenditure and locomotor activity (D-amphetamine (Bushnell and Gordon, 1987)). From this comparison, it is clear that in the case of sibutramine, the relation between activity and energy expenditure is more amphetamine-like, rather than DNP-like. More compounds and more detailed, preferably quantitative, characterization are necessary to use such relation as a tool of selection of successful drug candidates.

Acknowledgements

Authors would like to thank Mrs. B. E. Andreasen, Mrs. L. Kristensen and Mrs. J. Tofte-Jensen for excellent technical assistance, and Dr. K. Dahl for the help with metergoline experiments.

References

Antoniou K, Kafetzopoulos E, Papadopoulou-Daifoti Z, Hyphantis T, Marselos M. D-amphetamine, cocaine and caffeine: a comparative study of acute effects on locomotor activity and behavioural patterns in rats. Neurosci Biobehav Rev 1998;23:189–96.

Balcioglu A, Wurtman RJ. Sibutramine, a serotonin uptake inhibitor, increases dopamine concentrations in rat striatal and hypothalamic extracellular fluid. Neuropharmacology 2000;39:2352–9.

Brown M, Bing C, King P, Pickavance L, Heal D, Wilding J. Sibutramine reduces feeding, body fat and improves insulin resistance in dietary-obese male Wistar rats independently of hypothalamic neuropeptide Y. Br J Pharmacol 2001;132:1898–904.

Bushnell PJ, Gordon CJ. Effects of D-amphetamine on behavioral and autonomic thermoregulation in mice. Pharmacol Biochem Behav 1987;27:431–5.

Casado A, Rodriguez VM, Portillo MP, Macarulla MT, Abecia LC, Echevarria E, Casis L. Sibutramine decreases body weight gain and increases energy expenditure in obese Zucker rats without changes in NPY and orexins. Nutr Neurosci 2003;6:102–11.

Connoley IP, Liu YL, Frost I, Reckless IP, Heal DJ, Stock MJ. Thermogenic effects of sibutramine and its metabolites. Br J Pharmacol 1999;126:1487–95.

Day C, Bailey CJ. Effect of the antiobesity agent sibutramine in obese-diabetic *ob*/*ob* mice. Int J Obes Relat Metab Disord 1998;22:619–23.

Finer N. Sibutramine: its mode of action and efficacy. Int J Obes 2002;26:S29–33.

Giordano A, Centemeri C, Zingaretti MC, Cinti S. Sibutramine-dependent brown fat activation in rats: an immunohistochemical study. Int J Obes 2002;26:354–60.

Glick SD, Haskew RE, Maisonneuve IM, Carlson JN, Jerussi TP. Enantioselective behavioral effects of sibutramine metabolites. Eur J Pharmacol 2000;397:93-102.

Hansen DL, Toubro S, Stock MJ, Macdonald IA, Astrup A. Thermogenic effects of sibutramine in humans. Am J Clin Nutr 1998;68:1180–6.

Hansen DL, Toubro S, Stock MJ, Macdonald IA, Astrup A. The effect of sibutramine on energy expenditure and appetite during chronic treatment without dietary restriction. Int J Obes 1999;23:1016–24.

Harper JA, Dickinson K, Brand MD. Mitochondrial uncoupling as a target for drug development for the treatment of obesity. Obes Rev 2001;2:255–65.

Jackson HC, Needham AJ, Hutchins LJ, Mazurkiewicz SE, Heal DJ. Comparison of the effects of sibutramine and other monoamine reuptake inhibitors on food intake in the rat. Br J Pharmacol 1997;121:1758–62.

Keesey RE, Corbett SW. Adjustments in daily energy expenditure to caloric restriction and weight loss by adult obese and lean Zucker rats. Int J Obes 1990;14:1079–84.

Lahdesmaki J, Sallinen J, MacDonald E, Sirvio J, Scheinin M. alpha2-Adrenergic drug effects on brain monoamines, locomotion, and body temperature are largely abolished in mice lacking the alpha2A-adrenoceptor subtype. Neuropharmacology 2003;44:882–92.

Lazzer S, Boirie Y, Montaurier C, Vernet J, Meyr M, Vermorel M. A weight reduction program preserves fat-free mass but not metabolic rate in obese adolescents. Obes Res 2004;12:233–40.

Levin BE, Dunn-Meynell AA. Sibutramine alters the central mechanisms regulating the defended body weight in diet-induced obese rats. Am J Physiol Regul Integr Comp Physiol 2000;279:R2222–8.

Liu YL, Connoley IP, Harrison J, Heal DJ, Stock MJ. Comparison of the thermogenic and hypophagic effects of sibutramine's metabolite 2 and other monoamine reuptake inhibitors. Eur J Pharmacol 2002a;452:49–56.

Liu YL, Heal DJ, Stock MJ. Mechanism of the thermogenic effect of Metabolite 2 (BTS 54 505), a major pharmacologically active metabolite of the novel anti-obesity drug, sibutramine. Int J Obes 2002b;26:1245–53.

Luque CA, Rey JA. The discovery and status of sibutramine as an anti-obesity drug. Eur J Pharmacol 2002;440:119–28.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.

Nakagawa T, Ukai K, Ohyama T, Gomita Y, Okamura H. Effects of chronic administration of sibutramine on body weight, food intake and motor activity in neonatally monosodium glutamate-treated obese female rats: relationship of antiobesity effect with monoamines. Exp Anim 2000;49:239–49.

Nisoli E, Carruba MO. An assessment of the safety and efficacy of sibutramine, an anti-obesity drug with a novel mechanism of action. Obes Rev 2000;1:127–39.

Rowley HL, Butler SA, Prow MR, Dykes SG, Aspley S, Kilpatrick IC, et al. Comparison of the effects of sibutramine and other weight-modifying drugs on extracellular dopamine in the nucleus accumbens of freely moving rats. Synapse (New York) 2000;38:167–76.

Seagle HM, Bessesen DH, Hill JO. Effects of sibutramine on resting metabolic rate and weight loss in overweight women. Obes Res 1998;6:115–21.

Starling RD, Liu XL, Sullivan DH. Influence of sibutramine on energy expenditure in African American women. Obes Res 2001;9:251–6.

Stock MJ. Effects of low (LCD) and very low (VLCD) energy diets on metabolic rate and body composition in obese (*fa*/*fa*) Zucker rats. Int J Obes 1989;13:61–5.

Stock MJ. Sibutramine: a review of the pharmacology of a novel anti-obesity agent. Int J Obes 1997;21(Suppl 1):S25–9.

Strack AM, Shu J, Camacho R, Gorski JN, Murphy B, MacIntyre DE, et al. Regulation of body weight and carcass composition by sibutramine in rats. Obes Res 2002;10:173–81.

Stricker-Krongrad A, Souquet A, Burlet C. Effects of sibutramine on feeding behavior in obese and lean Zucker rats. Int J Obes Relat Metab Disord 1995;19(suppl 2):399.

Zwiauer KF, Mueller T, Widhalm K. Resting metabolic rate in obese children before, during and after weight loss. Int J Obes Relat Metab Disord 1992;16:11–6.