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Thermogenic effect of YM348, a novel 5-HT_{2C}-receptor agonist, in rats

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Abstract

We have investigated the effect of *S*-2-(7-ethyl-1H-furo[2,3-g]indazol-1-yl)-1-methylethylamine (YM348), a novel 5-HT_{2C}-receptor agonist, on body temperature and energy expenditure in Wistar rats. m-Chlorophenylpiperazine (mCPP) and *S*-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine (RO 60-0175) were used as reference 5-HT_{2C}-receptor agonists. Administration of YM348, mCPP and RO 60-0175 dose-dependently and significantly increased body temperature in rats. YM348- or RO 60-0175-induced hyperthermia was significantly attenuated by the non-selective 5-HT_2-receptor antagonist methysergide and the selective 5-HT_{2C}-receptor antagonist SB242084, but not by the selective 5-HT_{2A}-receptor antagonist MDL100907. mCPP-induced hyperthermia was significantly attenuated by methysergide, SB242084 and MDL100907. In addition to the increase in body temperature, YM348, mCPP and RO 60-0175 produced dose-related and significant increases in energy expenditure. YM348-, mCPP- and RO 60-0175-induced increases in energy expenditure were significantly attenuated by methysergide and SB242084 but not by MDL100907. These results suggested that 5-HT_{2C}-receptor stimulation increased body temperature and energy expenditure and that the 5-HT_{2C} receptor was the target receptor in the thermogenic effect of YM348 in Wistar rats.

Introduction

Body weight is regulated by complex interrelationships between energy availability, determined by appetite control or food absorption limitation, and thermogenesis or lipolysis, determined by effects on fat deposition or white adipose tissue mass. Recent progress has provided important new insights into how these central and peripheral components of weight control are mediated by receptors for several key neurotransmitters and hormones. Serotonin (5-hydroxytryptamine, 5-HT) is considered as one of the most important neurotransmitters in the central control of body weight regulation (Wozniak et al 1988, 1989). Serotonin mediates its effects through at least 14 different subtypes of 5-HT receptor which are classified into seven major families termed 5-HT_1 to 5-HT_7 (Boess & Martin 1994; Martin & Humphrey 1994). Of the many kinds of 5-HT-receptor subtype, the 5-HT_{2C} receptor is thought to contribute to anorectic properties and hyperthermia. The high-affinity 5-HT_{2C}-receptor agonist m-chlorophenylpiperazine (mCPP), a metabolite of the antidepressant trazodone, is known to decrease food intake in rats (Kennett & Curzon 1988a, b). In a study in 5-HT_{2C}-receptor knockout mice, m-CPP failed to show a hypophagic effect, indicating that the 5-HT_{2C} receptor is the primary site at which mCPP exerts its appetite-suppressant action. In addition to hypophagia, mCPP is also reported to induce hyperthermia (Mazzola-Pomietto et al 1996). Given the close relationship between body temperature and energy consumption, we considered it likely that the 5-HT_{2C} receptor is also involved in energy expenditure in terms of body temperature regulation. However, it has been unclear whether stimulation of the 5-HT_{2C} receptor actually increases energy expenditure. A further complication is that mCPP is reported to have poor 5-HT_{2C} receptor selectivity (Barnes & Sharp 1999). Any examination of the involvement of the 5-HT_{2C} receptor in mechanisms of energy expenditure and temperature control therefore requires the use of more selective 5-HT_{2C}-receptor agonists.

YM348 (*S*-2-(7-ethyl-1H-furo[2,3-g]indazol-1-yl)-1-methylethylamine) is a novel and orally active 5-HT_{2C}-receptor agonist, proven to be more selective to the 5-HT_{2C}

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receptor than mCPP (Kimura et al 2004). The purpose of this study was to investigate the effect of YM348 and two other 5-HT_{2C}-receptor agonists on body temperature and energy expenditure in Wistar rats.

Materials and Methods

Animals

Adult male Wistar rats (200–350 g; Japan Charles River, Inc.) were housed in an animal room maintained at $23 \pm 1°C$ with a 12-h light cycle (lights on 0730 h). The animals arrived at the animal department more than one week before the start of the experiments. They were maintained on a conventional pelleted stock diet, and fasted overnight before experiments. Each rat was moved to an individual cage at least 2 h before each experiment began for acclimation to the laboratory environment. Experiments were conducted between 0830 and 1630 h and were performed in accordance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical.

Drugs

Three kinds of 5-HT_{2C}-receptor agonist were used. *S*-2-(7-ethyl-1H-furo[2,3-g]indazol-1-yl)-1-methylethylamine (YM348 hemisuccinate) and *S*-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine (RO 60-0175) were synthesized at Yamanouchi Pharmaceutical Co., Ltd. m-Chlorophenylpiperazine (mCPP-hydrochloride) was purchased from Tocris Co., Ltd. The doses of YM348, mCPP and RO60–0175 were expressed in terms of the free form. All compounds were orally administered by dissolving in sterile water at a concentration designed to provide the appropriate volume of $5\,mL\,kg^{-1}$ body weight. Doses of YM348, RO 60-0175 and mCPP were selected on the basis of previous studies (Wozniak et al 1989; Mazzola-Pomietto et al 1996; Clifton et al 2000; Kennett et al 2000; Kimura et al 2004) and our pilot studies.

MDL100907, a 5-HT_{2A}-receptor-selective antagonist, was synthesized at Yamanouchi Pharmaceutical Co., Ltd and SB242084, a 5-HT_{2C}-receptor-selective antagonist, and methysergide, a 5-HT_2-receptor-nonselective antagonist, were purchased from Sigma. Co., Ltd. All drugs were prepared as solutions in physiological saline containing drops of Tween 80. MDL100907 was administrated subcutaneously at a dose of $0.1\,mg\,kg^{-1}$. SB242084 and methysergide were given intraperitoneally at 10 and $3\,mg\,kg^{-1}$, respectively. Each antagonist was given 30 min before agonist administration. Doses of methysergide, SB242084 and MDL100907 were chosen primarily on the basis of previous studies (Griebel et al 1997; Clifton et al 2000; Kennett et al 2000; Vickers et al 2001; Lavich et al 2003) and of our pilot studies conducted to demonstrate their antagonistic effects. The effect of antagonists on 5-HT_{2C} agonist-induced temperature and energy expenditure change was observed at the time point when each agonist showed the maximum effect.

Measurement of body temperature

Rectal temperature was measured with a rectal thermometer probe for freely moving animals (Physitemp, Model BAT-12; Physitemp Instruments Inc., USA) in a room maintained at $23 \pm 1°C$. The probe was inserted approximately 3 cm and held until a maximum plateau temperature had been recorded. Pre-administration temperature was measured 1 h before 5-HT_{2C}-receptor antagonist or agonist administration. Post-administration temperature was measured at 1, 2, 3 and 4 h after the administration of 5-HT_{2C}-receptor agonists. The temperature changes from pre- to post-administration were taken as the respective values.

Measurement of energy expenditure

Energy expenditure was measured by an open-circuit indirect calorimetry system (Oxymax, Model V.5.30; Columbus Inc.; OH). Oxygen and carbon dioxide concentrations in outgoing air from four different cages were measured successively. Air from each cage was sampled every 15 min during the experimental period following administration of agonists. Respiratory O_2 uptake (VO_2; $mL\,kg^{-1}\,min^{-1}$) and CO_2 production (VCO_2; $mL\,kg^{-1}\,min^{-1}$) from each animal was measured, expended energy was then calculated with the Oxymax computer and expressed as $kcal\,min^{-1}$. The average of values measured 45 and 60 min before 5-HT_{2C}-receptor agonist or antagonist administration was used as the pre-administration value. The average of values at 45 and 60 min of each hour following 5-HT_{2C}-receptor agonist administration was used to plot the time-course of responses. Measurement continued until 4 h after administration and the increase in calories for each rat was determined by comparison with the base value of each animal.

Statistics

The results are presented as the means ± s.e.m. Rectal temperature and energy expenditure in the 4-h study were analysed by two-way analysis of variance where drug was a between-subject factor and time a within-subject factor. Significant interactions were succeeded by the performance of one-way analysis of variance at each level of time. Dunnett's multiple range test was used where appropriate. Rectal temperature and energy expenditure in the antagonist study were analysed by one-way analysis of variance followed by Student's *t*-test or Dunnett's multiple range test. A *P*-value less than 0.05 was considered statistically significant.

Results

Effect of YM348, mCPP and RO 60-0175 on rectal temperature

The basal rectal temperature of the control group was $36.5 \pm 0.2°C$. Oral administration of YM348 (0.1, 0.3, 1 or $3\,mg\,kg^{-1}$), mCPP (1, 3, 10 or $30\,mg\,kg^{-1}$) or RO 60-0175 (1, 3 or $10\,mg\,kg^{-1}$) increased body temperature in a dose-dependent manner (significant drug × time interaction,

$F_{(16,144)} = 6.3$ $P < 0.01$, $F_{(16,148)} = 3.8$ $P < 0.01$ and $F_{(12,112)} = 3.2$ $P < 0.01$, respectively). For YM348, the time of maximum hyperthermia occurred 1 h after administration (Figure 1a). The statistically significant rectal temperature changes of 1.4 ± 0.2 and $1.7 \pm 0.2\,\Delta^{\circ}C$ were observed 1 h after administration of 1 and 3 mg kg^{-1} YM348, respectively (analysis of variance: $F_{(4,36)} = 11.2$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$ and $P < 0.01$, respectively). The time of maximum hyperthermia induced by mCPP also occurred 1 h after administration (Figure 1b). The significant rectal temperature changes of 0.7 ± 0.2, 0.8 ± 0.2, 1.0 ± 0.2 and $1.2 \pm 0.2\,\Delta^{\circ}C$ were observed 1 h after administration of 1, 3, 10, and 30 mg kg^{-1} mCPP, respectively (analysis of variance: $F_{(4,37)} = 8.2$ $P < 0.01$, Dunnett's multiple range test: $P < 0.05$, $P < 0.01$, $P < 0.01$ and $P < 0.01$, respectively). The time of maximum hyperthermia induced by RO 60-0175 occurred 2 h after administration (Figure 1c). The significant rectal temperature changes of 1.2 ± 0.1 and $1.4 \pm 0.4\,\Delta^{\circ}C$ was observed 2 h after administration of 3 and 10 mg kg^{-1} RO 60-0175, respectively (analysis of variance: $F_{(3,28)} = 12.8$ $P < 0.01$, Dunnett's multiple range test: $P < 0.05$ and $P < 0.01$, respectively).

Effect of antagonists on YM348-, mCPP- and RO 60-0175-induced temperature change

Methysergide and SB242084 at 3 and 10 mg kg^{-1}, respectively, significantly inhibited the hyperthermia induced by 1 mg kg^{-1} YM348 by 87.4% and 80.7%, respectively (analysis of variance: $F_{(4,42)} = 10.2$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$ and $P < 0.01$, respectively). In contrast, MDL100907 did not inhibit YM348-induced hyperthermia (Figure 2a). Methysergide and SB242084 at 3 and 10 mg kg^{-1}, respectively, significantly inhibited the temperature change induced by 3 mg kg^{-1} RO 60-0175 by 86.6% and 107.5%, respectively (analysis of variance: $F_{(4,35)} = 14.0$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$ and $P < 0.01$, respectively). However, 0.1 mg kg^{-1} MDL100907 did not inhibit RO 60-0175-induced hyperthermia (Figure 2c). Methysergide, SB242084 and MDL100907 significantly inhibited the hyperthermia induced by 10 mg kg^{-1} mCPP by 157.1%, 87.3% and 60.3%, respectively (analysis of variance: $F_{(4,35)} = 9.9$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively) (Figure 2b). Methysergide, SB242084 and MDL100907 alone did not effect the rectal temperature (-0.1 ± 0.3, -0.2 ± 0.1, and $-0.1 \pm 0.2\,\Delta^{\circ}C$, respectively: no significant difference from control group by analysis of variance ($F_{(3,20)} = 1.5$ $P > 0.05$)).

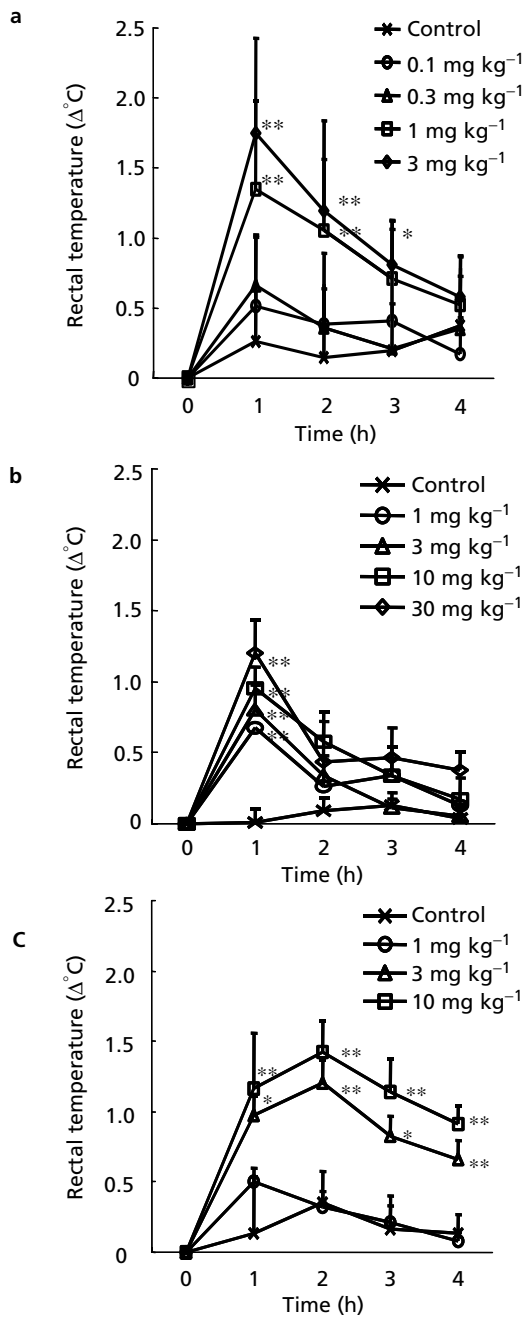

Figure 1 Effect of YM348, mCPP and RO 60-0175 on rectal temperature for 4 h after oral administration in rats. Rectal temperature was increased by administration of YM348 (a), mCPP (b) and RO 60-0175 (c). All values represent the mean ± s.e.m. for 8–12 animals. *$P < 0.05$, **$P < 0.01$, significantly different to control values by two-way repeated analysis of variance followed by Dunnett's multiple range test.

Effect of YM348, mCPP and RO 60-0175 on energy expenditure

The basal energy expenditure of the control group was 0.025 ± 0.001 kcal min^{-1}. Oral administration of YM348, mCPP or RO 60-0175 produced dose-related increases in energy expenditure (significant drug × time interaction, $F_{(12,96)} = 2.3$ $P < 0.05$, $F_{(16,140)} = 3.4$ $P < 0.01$ and $F_{(12,112)} = 4.2$ $P < 0.01$, respectively). For YM348, the time of maximum energy expenditure occurred 1 h after administration (Figure 3a). YM348 at 3 and 10 mg kg^{-1} induced significant increases in energy expenditure of 0.005 ± 0.001 and 0.004 ± 0.001 kcal min^{-1}, respectively, 1 h after administration (analysis of variance: $F_{(3,28)} = 7.6$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$ and

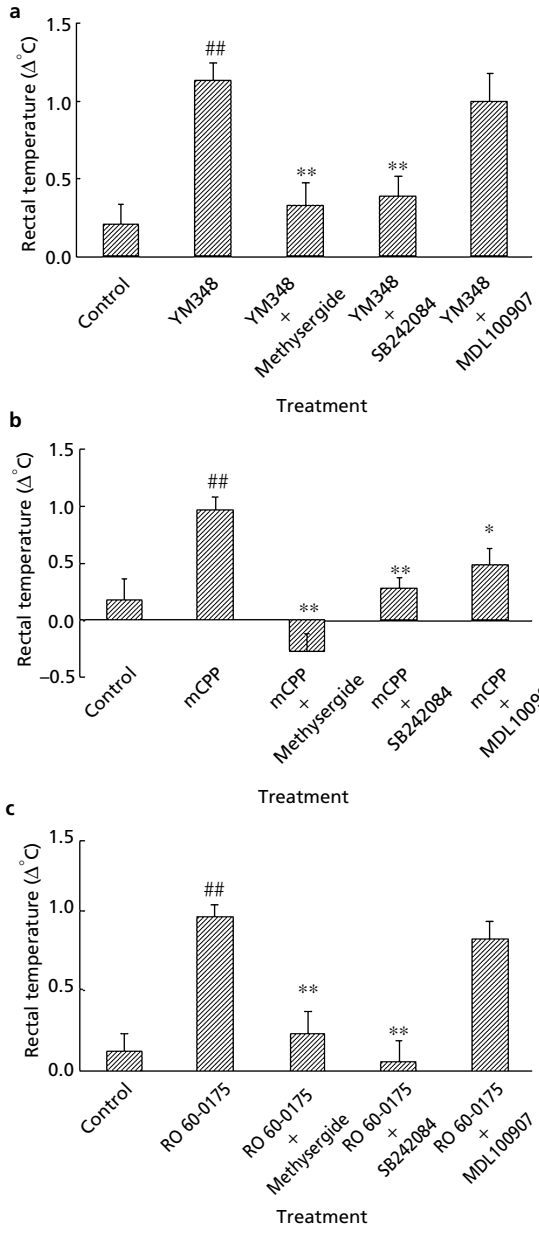

Figure 2 Effect of methysergide, SB 242084 and MDL100907 on YM348-, mCPP- and RO 60-0175-induced hyperthermia in rats. Vehicle, methysergide (3 mg kg^{-1}, i.p.), SB 242084 (10 mg kg^{-1}, i.p.) or MDL100907 (0.1 mg kg^{-1}, s.c.) was given 30 min before distilled water, YM348 (1 mg kg^{-1}, p.o.), mCPP (10 mg kg^{-1}, p.o.) or RO 60-0175 (3 mg kg^{-1}, p.o.). All columns represent the mean ± s.e.m. for 8–10 animals. Significantly different from the (vehicle + distilled water)-treated group, $^{\#\#}P < 0.01$ by analysis of variance followed by Student's *t*-test, and from the (vehicle + YM348, mCPP or RO 60-0175)-treated group, $*P < 0.05$, $**P < 0.01$ by analysis of variance followed by Dunnett's multiple range test.

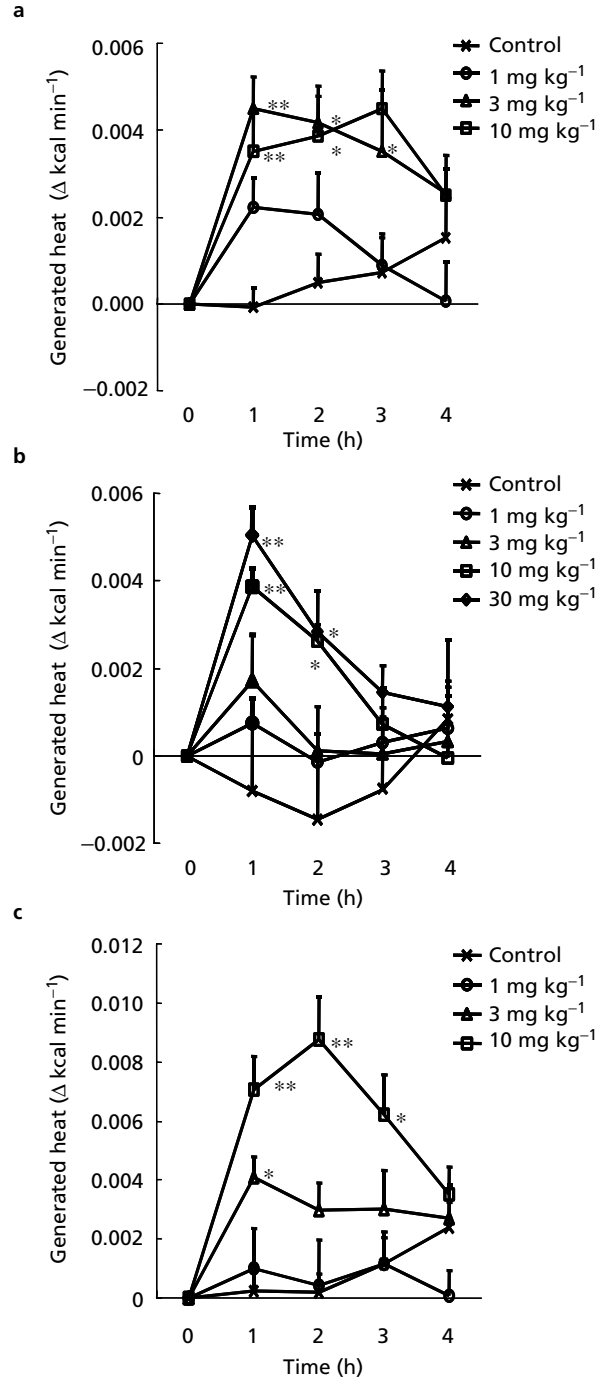

Figure 3 Effect of YM348, mCPP and RO 60-0175 on calorie expenditure for 4 h after administration in rats. Energy expenditure was increased by administration of YM348 (a), mCPP (b) and RO 60-0175 (c). All values represent the mean ± s.e.m. for 8–12 animals. $*P < 0.05$, $**P < 0.01$, significant difference to vehicle values by two-way repeated analysis of variance followed by Dunnett's multiple range test.

$P < 0.01$, respectively). The time of maximum energy expenditure induced by mCPP also occurred 1 h after administration (Figure 3b). At this time, 10 and 30 mg kg^{-1} mCPP induced significant increases in energy expenditure of 0.004 ± 0.000 and 0.005 ± 0.001 kcal min^{-1}, respectively (analysis of variance: $F_{(4,35)} = 6.3$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$ and $P < 0.01$, respectively). The time of maximum energy expenditure

induced by RO 60-0175 occurred 2 h after administration (Figure 3c). RO 60-0175 at a dose of 10 mg kg^{-1} induced a significant increase in energy expenditure of 0.009 ± 0.004 kcal min^{-1} at the time of maximum effect (analysis of variance: $F_{(3,28)} = 11.2$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$ and $P < 0.01$, respectively).

Effect of antagonists on YM348-, mCPP- and RO 60-0175-induced energy expenditure

Methysergide and SB242084 significantly inhibited the increase in energy expenditure by 65.0% and 80.7%, respectively, 1 h after administration of 3 mg kg^{-1} YM348 (analysis of variance: $F_{(4,36)} = 4.6$ $P < 0.01$, Dunnett's multiple range test: $P < 0.05$ and $P < 0.05$, respectively). However, 0.1 mg kg^{-1} MDL100907 did not inhibit the increase in energy expenditure induced by YM348 (Figure 4a). Methysergide and SB242084 significantly inhibited the increase in energy consumption 1 h after 10 mg kg^{-1} mCPP, by 109.1% and 75.5%, respectively (analysis of variance: $F_{(4,35)} = 5.1$ $P < 0.01$, Dunnett's multiple range test: $P < 0.01$ and $P < 0.05$, respectively). However, 0.1 mg kg^{-1} MDL100907 did not inhibit the increase in energy consumption induced by mCPP (Figure 4b). Methysergide and SB242084 significantly inhibited the increase in energy consumption 2 h after 10 mg kg^{-1} RO 60-0175 by 72.2% and 96.1%, respectively (analysis of variance: $F_{(4,35)} = 5.5$ $P < 0.01$, Dunnett's multiple range test: $P < 0.05$ and $P < 0.01$, respectively). However, MDL100907 did not inhibit the increase in energy consumption induced by RO 60-0175 (Figure 4c). Methysergide, SB242084 or MDL100907 alone did not effect the energy expenditure (-0.000 ± 0.002, -0.001 ± 0.001, and -0.001 ± 0.002 Δ kcal min^{-1}, respectively: no significant difference from control group by analysis of variance ($F_{(3,18)} = 0.63$ $P > 0.05$)).

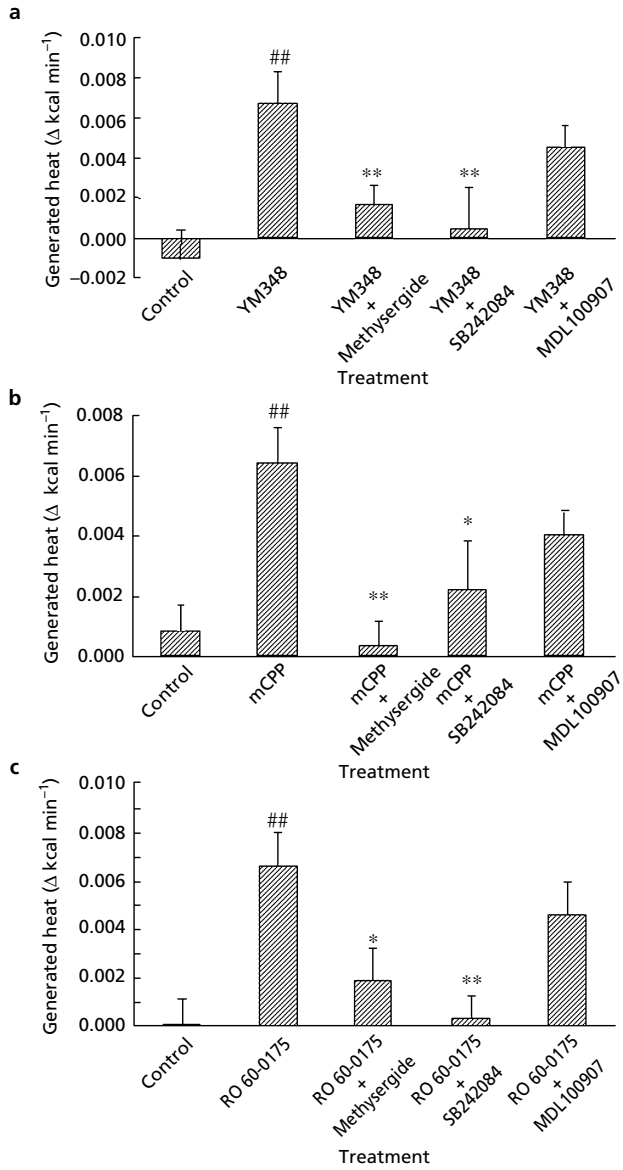

Figure 4 Effect of methysergide, SB 242084 and MDL100907 on YM348-, mCPP- or RO 60-0175-induced energy expenditure in rats. Vehicle, methysergide (3 mg kg^{-1}, i.p.), SB 242084 (10 mg kg^{-1}, i.p.), or MDL100907 (0.1 mg kg^{-1}, s.c.) was given 30 min before distilled water, YM348 (3 mg kg^{-1}, p.o.), mCPP (10 mg kg^{-1}, p.o.) or RO 60-0175 (10 mg kg^{-1}, p.o.). All columns represent the mean $\pm$ s.e.m. for 8–10 animals. Significantly different from the (vehicle + distilled water)-treated group, $^{\#\#}P < 0.01$ by analysis of variance followed by Student's *t*-test, and from the (vehicle + YM348, mCPP or RO 60-0175)-treated group, $^{*}P < 0.05$, $^{**}P < 0.01$ by analysis of variance followed by Dunnett's multiple range test.

Discussion

The results unequivocally demonstrated the effects of the 5-HT$_{2C}$-receptor agonist YM348 and those of two other 5-HT$_{2C}$-receptor agonists on body temperature and energy expenditure in rats. The dose-dependent effects of YM348, mCPP and RO 60-0175 on the increase of body temperature were evident in the first experiment (Figure 1). Although these 5-HT$_{2C}$-receptor agonists were administered at doses that were relatively high compared with the doses at which they produce their other effects, such as penile erection (Kimura et al 2004), the almost complete blockage of their effects by pre-dosing with the 5-HT$_{2C}$ receptor-selective antagonist SB242084 (Figure 2) confirmed that the 5-HT$_{2C}$ receptor, and not the 5-HT$_{2A}$ receptor mediated the YM348- and RO 60-0175-induced increases in body temperature. In the case of mCPP, its hyperthermic effect was antagonized not only by SB242084, but also by a 5-HT$_{2A}$ receptor-selective antagonist, MDL100907. mCPP was reported to show affinity for the 5-HT$_{2A}$ receptor as well as the 5-HT$_{2C}$ receptor (Barnes & Sharp 1999) and partial-agonistic activity on the 5-HT$_{2A}$ receptor (Kimura et al 2004). Considering that 5-HT$_{2A}$-receptor agonists are reported to induce hyperthermia (Salmi & Ahlenius 1998), it is possible that 5-HT$_{2A}$ receptors as well as 5-HT$_{2C}$ receptors were involved in the mechanism of hyperthermia induced by mCPP.

Energy expenditure is thought to have a close relationship with body temperature, and so it would seem that the 5-HT_{2C} receptor is involved in energy expenditure as well as body temperature. Indeed, dose-dependent increases in energy expenditure by stimulation of 5-HT_{2C} receptors were observed in this study using YM348, mCPP and RO 60-0175 (Figure 3). Nonogaki et al (2002) reported that oxygen consumption was significantly decreased in 5-HT_{2C} receptor mutant mice compared with wild-type animals. Interestingly, in our study, mCPP increased body temperature via 5-HT_{2A} and 5-HT_{2C} receptors, and increased energy expenditure via the 5-HT_{2C} receptor alone, without the 5-HT_{2A} receptor. It was therefore possible that the increase in body temperature by 5-HT_{2C}-receptor agonists was caused by increased energy expenditure resulting from 5-HT_{2C} receptor stimulation.

The sites of action of 5-HT_{2C}-receptor agonists on energy expenditure were not addressed in this study and remain largely unknown, since 5-HT_{2C} receptors are widely distributed throughout the brain (Zifa & Fillion 1992). A possible mechanism for 5-HT_{2C} receptor-mediated thermogenesis may be via central stimulation of the sympathetic system resulting in stimulation of thermogenic effectors such as brown adipose tissue (Sakaguchi & Bray 1989). On this basis, we suggest that the possible sites for the effects of 5-HT_{2C}-receptor agonists on thermogenesis are the spinal intermediolateral columns, hypothalamus and brain stem.

A study using 5-HT_{2C} receptor mutant mice has shown a number of interesting actions related to the 5-HT_{2C} receptor; 5-HT_{2C} receptor-deficient mice, for example, became obese as a result of abnormal control of feeding behaviour (Tecott et al 1995). Nonogaki et al (2002) showed that the 5-HT_{2C} receptor played a role in the control of appetite and also in gene expression related to energy expenditure. 5-HT_{2C} receptors in the brain may therefore play important roles in the central and in the peripheral energy expenditure control in the regulation of body weight.

In addition to the previously known hypophagic effect of 5-HT_{2C} receptor agonists, this study has confirmed the pharmacological importance of the 5-HT_{2C} receptor in thermogenesis and energy expenditure. 5-HT_{2C}-receptor agonists may therefore have great potential as anti-obesity drugs through their effects in increasing thermogenesis and energy expenditure and in decreasing food intake.

Conclusion

The results suggested that 5-HT_{2C}-receptor stimulation increased body temperature and energy expenditure, and that the 5-HT_{2C} receptor was the target receptor in the thermogenic effect of YM348 in Wistar rats.

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