Note

Mitigation of Obesity by BRL 26830A, a New β -Adrenoceptor Agonist, in MSG Obese Mice

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Summary Half of the mice in both the monosodium-L-glutamate (MSG)-induced obesity and saline control groups were given BRL 26830A via a gastric tube at a daily dose of 5 mg/kg for 2 weeks, and the other half given distilled water. BRL 26830A administration significantly increased guanosine-5'-diphosphate (GDP)-binding in brown adipose tissue (BAT) and the resting metabolic rate (RMR), and significantly reduced retroperitoneal white adipose tissue (WAT) pads in both groups. It also markedly reduced body weight in MSG obese mice that had reduced BAT thermogenesis and decreased RMR. However, food intake was unchanged in both groups. Neither β_1 - nor β_2 -selective antagonists affected the increase of RMR induced by BRL 26830A, but a non-selective β -antagonist completely inhibited its increase. These results suggest that BRL 26830A, which is a new β -adrenoceptor agonist, stimulates BAT thermogenesis, increases RMR, and reduces WAT, thus contributing to the mitigation of obesity.

Key Words β -adrenoceptor agonist (BRL 26830A), anti-obesity effect, brown adipose tissue (BAT), GDP-binding, resting metabolic rate (RMR), MSG obese mice, β_1 -selective antagonist, β_2 -selective antagonist

Brown adipose tissue (BAT) is the main effector of diet-induced thermogenesis (1) as well as non-shivering thermogenesis (2), and a defect in or an absence of BAT predisposes to obesity, as has been shown in various obese animals (3–5). Therefore, the activation of impaired BAT function in obese animals may lead to the mitigation of obesity (6, 7). In this study, we examined whether a new β -adrenoceptor agonist, BRL 26830A (Fig. 1) (6), could stimulate thermogenesis in BAT, by measuring BAT mitochondrial guanosine-5'-diphosphate (GDP)-binding (a thermogenic indicator) and the resting metabolic rate (RMR) in monosodium-L-glutamate (MSG)-induced obesity and saline control mice.

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Fig. 1. Chemical structure of BRL 26830A.

BAT temperature. Mice were placed in a box kept at $33 \pm 0.3^\circ\text{C}$. Thermocouples were inserted under the interscapular BAT pad and 3 cm into the rectum of twenty-five female CRJ-ICR mice (aged 12 weeks, weighing about 30 g, Charles River Japan, Tokyo) as described elsewhere (7). After a stable baseline had been achieved (about 30 min), 1-norepinephrine bitartrate (Sigma, U.S.A.) was injected intraperitoneally (i.p.) at a dose of 0.6 mg/kg. BRL 26830A (Beecham, U.K.), dissolved in distilled water, was given *via* a gastric tube at the doses of 1.0, 5.0, and 10.0 mg/kg. Distilled water only was given as a control. Temperatures were monitored for 60 min after the administration.

Effects of β_1 -, β_2 -selective, or non-selective β -adrenoceptor antagonists on RMR activation due to the administration of BRL 26830A. RMR was estimated using a closed-circuit metabolic system (Enviro-nics, U.S.A.) (8) in 5 groups of 6 female CRJ-ICR mice. One group was given BRL 26830A [5 mg/kg orally (p.o.)] alone, another was given BRL 26830A plus a β_1 -selective antagonist (Metoprolol, Sweden) (1 mg/kg, i.p.), and another group was given BRL 26830A plus a β_2 -selective antagonist (ICI 118551, ICI, U.K.) (1 mg/kg, i.p.). The other 2 groups had BRL 26830A plus a non-selective β -antagonist (DL-propranolol HCl, ICI, U.K.) (1 mg/kg, i.p.), or distilled water alone as a control.

RMR and GDP-binding In the MSG obese group, mice received a subcutaneous injection of 2 mg/kg (4) of MSG (Wako, Tokyo) daily for 5 days following birth and a control group was given injections of physiological saline. At 12 weeks after birth, half of the mice in both the MSG obesity and saline control groups were given BRL 26830A *via* a gastric tube at a daily dose of 5 mg/kg for 2 weeks, and the other half were given distilled water. After checking body weight and nasoanal length, RMR and BAT mitochondrial GDP-binding were measured in the BRL-26830A-treated and water-treated mice in both the MSG obesity and saline control groups. RMR was measured by the method mentioned above (8). For the measurement of mitochondrial GDP-binding in interscapular BAT, mice were killed by cervical dislocation at 0900 h. The BAT was rapidly removed, weighed, and homogenized in an ice-cold medium. The mitochondria were isolated and the protein content was measured. Mitochondrial GDP-binding was determined by the method of Nicholls (9). Statistical analysis was performed using Student's *t*-test.

Fig. 2. The mean changes in brown adipose tissue temperature (T_{BAT}) relative to core temperature (T_{core}) after the administration of 1-norepinephrine and BRL 26830A (5 mg/kg).

The interscapular BAT temperature (T_{BAT}) after the injection of 0.6 mg/kg of 1-norepinephrine was significantly ($p < 0.05$ – 0.01) higher than before injection. The mean changes in T_{BAT} relative to core temperature (T_{core}) were markedly ($p < 0.05$ – 0.01) higher from 4 to 60 min after injection. T_{BAT} and T_{core} after the administration of 1.0 mg/kg BRL 26830A were similar to those after water was given. T_{BAT} , after 5 mg/kg or 10 mg/kg of BRL 26830A, significantly ($p < 0.05$ – 0.01) increased from 2 to 60 min after administration. T_{core} after 10 mg/kg of BRL 26830A was markedly ($p < 0.05$ – 0.01) higher than that after water. Therefore, 5 mg/kg of BRL 26830A was used in the next experiment as the optimal dose. Figure 2 shows the mean changes with the standard error for T_{BAT} relative to T_{core} after the administration of 1-norepinephrine, BRL 26830A (5 mg/kg), and distilled water.

It has been widely (6) accepted that mammalian β -adrenoceptors exist as two subtypes, β_1 and β_2 , and rat brown adipocyte β -adrenoceptors have been classed as either β_1 or as a mixed β_1/β_2 population. The β_1 subtype predominates in the atria, whereas the β_2 subtype predominates in the trachea. A novel atypical β -adrenoceptor agonist, BRL 26830A, that exhibits low potency at classical β_1 and β_2 receptors, but selectively stimulates the atypical β -adrenoceptors in brown and white adipose tissues has been found (6). We investigated, first, whether or not BRL 26830A was affected by either a β_1 - or β_2 -selective antagonist. The administration of BRL 26830A (5 mg/kg, p.o.) increased RMR (ml/min $\cdot$ body weight^{0.75}) about twofold (27.3 ± 2.4) ($p < 0.02$) compared with water alone (15.2 ± 3.4). But the intraperi-

Table 1. Effects of BRL 26830A on body weight, lee index, food intake, R-WAT and I-BAT weights, I-BAT mitochondrial protein content, GDP-binding, and PMR in MSG obese and saline control mice.

	MSG		Saline	
	BRL 26830A (n=6)	Control (n=6)	BRL 26830A (n=6)	Control (n=6)
Body weight (g)	34.5 ± 1.9 ^{***}	46.2 ± 2.4 ^{**}	29.6 ± 0.9 [‡]	32.9 ± 1.2
Lee index	316.2 ± 4.8 ^{***}	369.4 ± 7.8 ^{**}	276.5 ± 6.6 [‡]	294.1 ± 7.2
Food intake (g/day)	3.6 ± 0.2 [*]	3.7 ± 0.3 [*]	5.0 ± 0.3	4.7 ± 0.3
R-WAT weight (g)	2.7 ± 0.6 ^{***}	5.6 ± 1.1 ^{**}	0.2 ± 0.1 [‡]	0.9 ± 0.1
I-BAT weight (mg)	165.1 ± 9.2 ^{***}	267.0 ± 16.8 ^{**}	90.3 ± 1.8	85.5 ± 4.4
I-BAT mitochondrial protein content (mg)	4.6 ± 0.4 [‡]	2.1 ± 0.3	3.6 ± 0.3 [‡]	2.2 ± 0.3
CDP-binding (pmol/mg · mitoprotein)	323.5 ± 32.9 [‡]	108.1 ± 19.2 ^{**}	398.0 ± 47.4 [‡]	185.4 ± 12.5
RMR (ml/min · BW ^{0.75})	20.8 ± 0.9 [‡]	9.6 ± 1.1 [*]	26.4 ± 2.2 [‡]	16.2 ± 3.9

Results are shown as mean ± SE. Lee index = $\frac{\sqrt[3]{\text{body weight (g)}}}{\text{nasoanal length (cm)}} \times 1,000$.

R-WAT, retroperitoneal white adipose tissue; I-BAT, interscapular brown adipose tissue. **p* < 0.05, ***p* < 0.01 vs. Saline; ‡*p* < 0.05, ‡‡*p* < 0.01 vs. Control.

toneal injection of β_1 - or β_2 -selective antagonists did not affect the increase of RMR induced by BRL 26830A (24.5 ± 3.1 or 25.4 ± 3.7 , respectively), while the non-selective β -antagonist completely inhibited the increase (16.6 ± 1.5). This result is consistent with a previous report (6), that this agent is neither β_1 - nor β_2 -selective. Second, we examined whether this agent could stimulate BAT function and increase RMR, resulting in the mitigation of obesity in MSG obese mice with reduced BAT function and decreased RMR (4). As shown in Table 1, the body weight, Lee index, retroperitoneal white adipose tissue, and interscapular BAT of obese mice were significantly greater than those of the controls. BRL 26830A significantly reduced these parameters in both groups except BAT weight in the control group. Interscapular BAT mitochondrial protein content was similar in the obese and control groups, but BRL 26830A significantly increased the content in both groups. GDP-binding and RMR in the obese group were significantly lower than in the controls, but BRL 26830A significantly increased these parameters in both groups. These results are in accordance with the previous reports using ob/ob mice (6, 10) and using fatty rats (10).

Recent studies with BRL 26830A have shown that the BAT is not the only site where stimulation of β -adrenoceptors leads to thermogenesis, and have pointed to skeletal muscles as an additional site (11). This has raised the possibility that BRL

26830A may be a useful drug in the treatment of obesity in human (12), even though the energetic relevance of BAT in human adults is less than in rodents (13). Furthermore, some reports show that BRL 26830A has an anti-hyperglycemic effect (10, 14). Therefore, it is possible that this agent will become a useful drug for the treatment of non-insulin-dependent diabetic patients who have both obesity and diabetes.

Our results suggest that BRL 26830A is a novel atypical β -adrenoceptor agonist that is neither β_1 - nor β_2 -selective, and that this agent stimulates BAT thermogenesis, increases RMR, and reduces white adipose tissue in MSG obese mice, which contributes to the mitigation of obesity.

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