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The Novel Neuropeptide Y Y_1 Receptor Antagonist J-104870: A Potent Feeding Suppressant with Oral Bioavailability

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Neuropeptide Y (NPY) is known to induce robust feeding through the action of NPY receptors in the hypothalamus. Among the subtypes of NPY receptors, Y_1 receptors may play a key role in feeding regulation. In the present study, we demonstrated that a novel Y_1 antagonist, J-104870, shows high selectivity and potency for the Y_1 receptor with an anorexigenic effect on NPY-mediated feeding. J-104870 displaced [^{125}I]peptide YY (PYY) binding to cloned human and rat Y_1 receptors with K_i values of 0.29 and 0.54 nM, respectively, and inhibited the NPY (10 nM)-induced increase in intracellular calcium levels (IC_{50} = 3.2 nM) in cells expressing human Y_1 receptors. In contrast, J-104870 showed low affinities for human Y_2 (K_i > 10 μM), Y_4 (K_i > 10 μM), and Y_5 receptors (K_i = 6 μM). In rat hypothalamic membranes, J-104870 also completely displaced the binding of [^{125}I]1229U91, which is known to bind to the typical Y_1 receptor, with a high affinity (K_i = 2.0 nM). Intracerebroventricular (ICV) injection of J-104870 (200 μg) significantly suppressed NPY (5 μg)-induced feeding in satiated Sprague–Dawley rats by 74%. Furthermore, ICV and oral administration of J-104870 (200 μg and 100 mg/kg, respectively) significantly suppressed spontaneous food intake in Zucker fatty rats. These findings suggested that J-104870 is a selective and potent nonpeptide Y_1 antagonist with oral bioavailability and brain penetrability. In addition, the anorexigenic effect of J-104870 clearly revealed the participation of the Y_1 receptor in NPY-mediated feeding regulation. The potent and orally active Y_1 antagonist J-104970 is a useful tool for elucidating the physiological roles of NPY in obesity.

The hypothalamus is a pivotal center for energy homeostasis of functions such as food intake, thermogenesis and fat accumulation. Several recent investigations have clearly shown that many factors are involved in the regulation of energy metabolism. Neuropeptide Y (NPY), the most potent orexigenic peptide identified thus far, is one of these factors. Chronic administration of NPY into the brain results in hyperphagia and body weight gain, reduces energy expenditure, and increases lipogenic activity in the liver and adipose tissues (1–6). It has also been reported that NPY-deficient *ob/ob* mice are less obese than *ob/ob* mice (7). These data suggest that NPY plays a crucial role in the control of body weight.

It is considered that NPY evokes several functions through the action of at least six distinct types of NPY receptors (8). Of these, we previously demonstrated that the Y_1 receptor is a key element in hypothalamic feeding regulation using the peptide Y_1 antagonist 1229U91 (9–11). A recent investigation using another Y_1 antagonist, BIBO3304, strongly supports our results (12). However, due to the limited activity of Y_1 antagonists after systemic injection, the extent of Y_1 participation in feeding regulation is still uncertain.

We showed in the present study that J-104870 is a new potent and selective antagonist for the Y_1 receptor with oral bioavailability and brain penetrability. Using this nonpeptide Y_1 antagonist, we investigated the participation of the Y_1 receptor in feeding regulation.

MATERIALS AND METHODS

Materials. Neuropeptide Y (NPY) was purchased from Peptide Institute (Osaka, Japan). Peptide YY (PYY) and pancreatic polypeptide (PP) were from Sigma (St. Louis, MO). [^{125}I]PYY and [^{125}I]PP were obtained from New England Nuclear-DuPont (Boston, MA). The culture reagents and bovine serum albumin (BSA) were from GIBCO (Grand Island, NY). All other chemicals were of analytical grade. J-104870 (6-(5-ethyl-1,3-thiazol-2-ylthiomethyl)-2-[3-methoxy-5-(2-propenyloxycarbonylamino)benzylamino]-4-morpholinopyridine) and 1229U91 (14) were synthesized by Banyu

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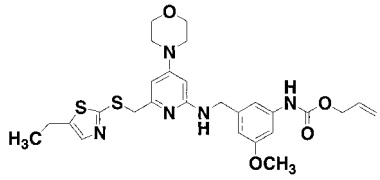

FIG. 1. Structure of J-104870.

Pharmaceutical Co., Ltd. The structure of J-104870 is shown in Fig. 1.

Cell culture. CHO-K1dhfr$^-$, LMtk$^-$, and COS-7 cells were obtained from ATCC (Rockville, MD). CHO-K1dhfr$^-$ cells expressing recombinant human Y_1, Y_2, and Y_4 receptors were grown in Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum (FBS), penicillin-G (100 IU/ml), streptomycin (100 μg/ml), and G418 (1 mg/ml). LMtk$^-$ cells expressing the recombinant human Y5 receptor were grown in Dulbecco's modified Eagle's medium (high glucose) with 10% FBS, penicillin-G (100 IU/ml), streptomycin (100 μg/ml), and G418 (0.8 mg/ml). COS-7 cells transiently expressing recombinant rat Y_1 receptors were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS, penicillin-G (100 IU/ml), and streptomycin (100 μg/ml). These cells were grown in a 95% air, 5% CO_2 humidified atmosphere at 37°C.

Binding experiments. Hypothalamus and cells were washed with 50 mM Hepes buffer (pH 7.4) containing 20% sucrose, homogenized, and centrifuged at 1000*g* for 15 min. The supernatant was centrifuged at 100,000*g* for 45 min. The pellets were resuspended in 5 mM Hepes buffer (pH 7.4) and centrifuged again. The membrane fraction was resuspended by a homogenizer in the same buffer and used for this study.

Binding of [^{125}I]PYY and [^{125}I]PP to membrane preparations was performed in 0.2 ml of 25 mM Tris buffer (pH 7.4) containing 10 mM $MgCl_2$, 1 mM PMSF, 0.1% bacitracin, and 0.5% BSA. The membranes (100–300 μg/ml) were incubated at 25°C for 120 min with [^{125}I]PYY (25 pM), [^{125}I]PP (25 pM), and [^{125}I]1229U91, respectively. Bound and free peptides were separated by filtration using a GF/C glass filter (Whatman, Tokyo, Japan) presoaked with 0.3% polyethylenimine. The remaining radioactivity on the filter was quantitated using a Cobra (Packard, Tokyo, Japan). Specific binding of [^{125}I]PYY, [^{125}I]PP, and [^{125}I]1229U91 was defined as the difference between total binding and nonspecific binding in the presence of 1 μM PYY, PP, and 1229U91, respectively.

Measurement of intracellular calcium ion concentrations. $[Ca^{2+}]_i$ was measured fluorometrically using a Ca^{2+}-sensitive fluorescent dye, fura-2. The cells expressing human NPY receptors were harvested using 0.25% trypsin and 0.02% EDTA. The cells (1.0×10^7 cells) were washed once with Krebs–Henseleit Hepes buffer containing 0.1% BSA (pH 7.4), suspended in 1 ml of the buffer, and incubated with 2 μM fura-2 acetoxymethylester at 37°C for 60 min. The fura-2-loaded cells were washed with the buffer and resuspended in 10 ml of the buffer. In a cuvette, 0.5 ml of the resultant suspension was stirred continuously at 37°C during the measurement. Test compounds or vehicle were added 5 min before the addition of NPY and the related ligands, and fluorescent intensity at an emission wavelength of 500 nm and excitation wavelengths of 340 and 380 nm was monitored with a CAF-110 intracellular ion analyzer (JASCO, Tokyo, Japan). $[Ca^{2+}]_i$ values were calculated according to a previously reported method (14).

In vivo experimental protocols. Male Zucker fatty rats (20–24 wks, Charles River Japan) and male Sprague–Dawley (SD) rats (7–8 wks, Charles River Japan) were used. The rats were housed in individual cages under controlled temperature (23 ± 2°C), humidity (55 ± 15%), and light–dark cycle (0700–1900). Water and pellets (CE-2, CLEA Japan) were available *ad libitum*.

In Experiment 1, SD rats were anesthetized with pentobarbital sodium (50 mg/kg ip; Dinabot), and a sterile 21-gauge guide cannula was implanted into the right lateral ventricle. At least 1 week after surgery, each rat was injected with either NPY (5 μg, $n = 10$) or NPY + J-104870 (50 μg or 200 μg, $n = 10$) and their food intake was monitored for 2 h. The volume of intracerebroventricular (ICV) injection was 10 μl. The injection was done between 0900 and 1130.

In Experiment 2, Zucker fatty rats were anesthetized with a ketamine (60 mg/kg ip; Sankyo, Tokyo, Japan) and chlorpromazine (6 mg/kg ip; Wako Pure Chemical, Osaka, Japan) mixture, and a 24-gauge guide cannula was implanted into the right lateral ventricle. At least 1 week after surgery, each group of 8–10 rats was injected with either vehicle or 200 μg of J-104870. The volume of ICV injection was 5 μl. The injection was done during the last hour of the light period, and spontaneous food intake was measured 2, 14, and 24 h after administration.

In Experiment 3, each group of 6 to 7 Zucker fatty rats was treated orally with 100 mg/kg/5 ml of J-104870 (suspended in 0.5% methylcellulose in distilled water) or vehicle around the last hour of the light period. Nocturnal and 24-h food consumption were measured.

All experimental procedures followed the Japanese Pharmacological Society Guideline for Animal Use. Results are given as means ± SE. Statistical analysis was performed using ANOVA followed by Bonferroni test.

RESULTS AND DISCUSSION

[^{125}I]PYY-specific binding to human and rat Y_1 receptors was inhibited by J-104870 with high affinities (K_i = 0.29 nM and 0.54 nM, respectively) (Table 1). In contrast, J-104870 showed low affinities for other cloned human NPY receptors such as Y_2, Y_4, and Y_5

TABLE 1

In Vitro Profiles of J-104870 for NPY Receptors

	Binding affinity (K_i, nM)						$[Ca^{2+}]$ response (IC_{50}, nM)
	hY_1	rY_1	hY_2 [^{125}I]PYY	hY_4[a]	hY_5	rY_1 (Hypothalamus) [^{125}I]1229U91	hY_1 10 nM NPY
hNPY	0.45	1.1	0.09	180	0.70	24	—
J-104870	0.26	0.51	>10000	>10000	6000	2.0	3.2

[a] [^{125}I]PP binding.

receptors (Table 1). J-104870 inhibited the NPY (10 nM)-induced $[Ca^{2+}]_i$ increase dose-dependently with an IC_{50} value of 3.2 nM in CHO-K1dhfr$^-$ cells expressing human Y_1 receptors (Table 1), while J-104870 alone did not induce a $[Ca^{2+}]_i$ increase even at a dose of 1 μM (data not shown). These findings showed that J-104870 is a highly potent and selective Y_1 antagonist.

We previously reported that [^{125}I]1229U91 selectively bound to the Y_1 receptor in rat hypothalamic membranes with a single class of binding site, while [^{125}I]PYY preferentially bound to the Y_2 receptor, which exists predominantly in the hypothalamus (9). J-104870 potently and completely displaced [^{125}I]1229U91 binding to the rat hypothalamic Y_1 receptor with a high affinity (K_i = 2.0 nM) (Table 1), although 10 μM J-104870 had no effect on [^{125}I]PYY binding to the membranes (data not shown). These results indicate that J-104870 selectively recognize the typical Y_1 receptor in rat hypothalamic membranes.

Intracerebroventricular injection of NPY (5 μg) induced rapid and robust feeding in satiated SD rats (Fig. 2). In comparison with the respective vehicles, J-104870 (200 μg alone) did not change the cumulative food intake, indicating that it had no effect on food intake in satiated rats (data not shown). Additionally, we did not observe any remarkable changes in other behaviors, including sedation or barrel-rolling, at any of tested doses. Simultaneous injection of J-104870 (200 μg) with NPY (5 μg) significantly suppressed food consumption induced by NPY by 74% in SD rats (Fig. 2). Furthermore, the 50-μg dose of J-104870 tended to attenuate feeding by 31% (Fig. 2). Taken together with the *in vitro* findings, J-104870 inhibited feeding through the inhibition of the Y_1 receptor, suggesting that the typical Y_1 receptor is involved in NPY-mediated feeding. These results were in agreement

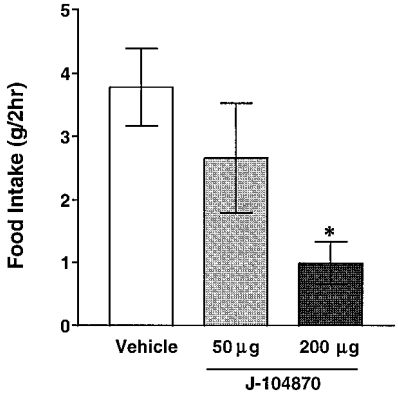

FIG. 2. Effect of intracebroventricular (ICV) coadministration of J-104870 on neuropeptide Y-induced food intake in Sprague-Dawley rats. *$P < 0.01$ compared with rats injected with NPY alone. The graph shows the cumulative food intake for 2 h after ICV injection of the drugs. Data are expressed as the mean ± SE. n = 9–11 rats/group (ANOVA followed by Bonferroni test).

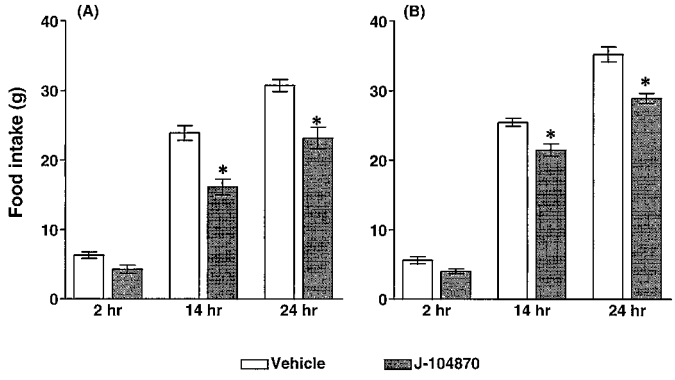

FIG. 3. Spontaneous food intake measured 2, 14, and 24 h after intracerebroventicular (A) and oral (B) administration of J-104870 in Zucker fatty rats. *$P < 0.01$ compared with rats treated with vehicle alone. The graph shows the cumulative food intake for 2, 14, and 24 h after administration of the drugs. Data are expressed as the mean ± SE. n = 9–11 rats/group (ANOVA followed by Bonferroni test).

with those of previous reports employing different types of Y_1 antagonists (9–12).

It is well known that the hypothalamic NPY level of Zucker fatty rats is remarkably increased compared with that of lean littermates and that this increase is accompanied by robust food intake (15). We previously demonstrated that a peptidic NPY antagonist, 1229U91, preferentially suppressed spontaneous feeding in obese littermates compared with lean littermates (10), indicating that Zucker fatty rats are sensitive to NPY antagonists. Intracerebroventricular (200 μg) and oral (100 mg/kg) administration of J-104870 in Zucker fatty rats significantly suppressed spontaneous feeding for 24 h by 25 and 18%, respectively (Fig. 3A). These findings indicate that the Y_1 receptor is involved in pathophysiological feeding in Zucker fatty rats. Thus, J-104870 is the first orally available and brain penetrable Y_1 antagonist. Even at 24 h after oral administration, 0.5 μM J-104870 in the brain was obtained.

Chronically injected NPY causes obesity due to increased energy intake and decreased energy expenditure (4–6). The present results using a structurally diverse, novel Y_1 antagonist strongly support the hypothesis that the Y_1 receptor plays a key role in energy intake regulated by NPY. However, there is limited information about the participation of the Y_1 receptor in energy expenditure. Orally available J-104870 should be a promising tool to address these issues.

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