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A Typical Y1 Receptor Regulates Feeding Behaviors: Effects of a Potent and Selective Y1 Antagonist, J-115814

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ABSTRACT

Neuropeptide Y (NPY) is a potent feeding stimulant. The orexigenic effect of NPY might be caused in part by the action of Y1 receptors. However, the existence of multiple NPY receptors including a possible novel feeding receptor has made it difficult to determine the relative importance of the Y1 receptor in feeding regulation. Herein we certified that the Y1 receptor is a major feeding receptor of NPY by using the potent and selective Y1 antagonist (−)-2-[1-(3-chloro-5-isopropyloxycarbonylaminophenyl)ethylamino]-6-[2-(5-ethyl-4-methyl-1,3-thiazol-2-yl)ethyl]-4-morpholinopyridine (J-115814) and Y1 receptor-deficient (Y1−/−) mice. J-115814 displaced ^{125}I-peptide YY binding to cell membranes expressing cloned human, rat, and murine Y_1 receptors with K_i values of 1.4, 1.8, and 1.9 nM, respectively, and inhibited NPY (10 nM)-induced increases in intracellular calcium levels via human Y1 receptors (IC_{50} = 6.8 nM). In contrast, J-115814 showed low affinities for human Y2 (K_i > 10 μM), Y4 (K_i = 640 nM) and Y5 receptors (K_i = 6000 nM). Intracerebroventricular (ICV) (10–100 μg) and intravenous (IV) (0.3–30 mg/kg) administration of J-115814 significantly and dose-dependently suppressed feeding induced by ICV NPY (5 μg) in satiated Sprague-Dawley rats. Intraperitoneal (IP) administration of J-115814 (3–30 mg/kg) significantly attenuated spontaneous feeding in db/db and C57BL6 mice. Feeding induced by ICV NPY (5 μg) was unaffected by IP-injected J-115814 (30 mg/kg) in Y1−/− mice and was suppressed in wild-type and Y5−/− mice. These findings clearly suggest that J-115814 inhibits feeding behaviors through the inhibition of the typical Y1 receptor. We conclude that the Y1 receptor plays a key role in regulating food intake.

Neuropeptide Y (NPY), a linear, 36-amino-acid peptide that belongs to a peptide family that also includes peptide YY (PYY) and pancreatic polypeptide (PP), is a potent feeding stimulant in the central nervous system (Tatemoto and Mutt, 1980; Tatemoto et al., 1982; Clark et al., 1984; Stanley and Leibowitz, 1984). Chronic administration of NPY into the brain results in hyperphagia and body weight gain (Stanley et al., 1986; Zarjevski et al., 1993). Concentrations of NPY and its mRNA in the hypothalamus are markedly increased during food deprivation and in genetically obese rodents (Guan et al., 1998; Kalra et al., 1991; Kesterson et al., 1997; Sanacora et al., 1990; White et al., 1990). In addition, NPY-deficient *ob*/*ob* mice are less obese than *ob*/*ob* mice and show a reduction in food intake (Erickson et al., 1996). From these data, it has been inferred that NPY is a major regulator of energy balance.

The presence of at least six distinct subtypes of NPY receptors has been described, and five of them (Y1, Y2, Y4, Y5, and Y6) have been cloned (Blomqvist and Herzog, 1997). Recent investigation showed that NPY-mediated feeding might be regulated by multiple subtypes of NPY receptors (Marsh et al., 1998; Pedrazzini et al., 1998; Inui, 1999; Kanatani et al., 2000b;). Of the subtypes, the Y1 receptor is considered to be a major feeding receptor because structurally diverse Y1 antagonists suppressed feeding behaviors (Daniels et al., 1995; Kanatani et al., 1996, 1998, 1999; Hipskind et al., 1997; Ishihara et al., 1998; Wieland et al., 1998). However, the possible existence of a novel subtype of feeding receptor has been reported (Kanatani et al., 2000b; O'Shea et al., 1997), which has made it more difficult to correctly address the role of the Y1 receptor in feeding regulation.

The participation of the Y1 receptor in ingestive behaviors is also supported by findings in Y1 deficient (Y1−/−) mice because exogenous NPY- and fasting-induced feeding is significantly suppressed in Y1−/− mice (Pedrazzini et al., 1998; Kanatani et al., 2000b). However, genetic deficiency of the Y1 receptor causes complete inactivation of the Y1 signals. Because the feeding paradigm is essential to life, compensation by other systems presumably occurs. Thus, it might be difficult to predict the precise physiological role of the Y1 receptor

ABBREVIATIONS: NPY, neuropeptide tyrosine; PYY, peptide tyrosine-tyrosine; PP, pancreatic polypeptide; BSA, bovine serum albumin; CHO, Chinese hamster ovary; FBS, fetal bovine serum; SD, Sprague-Dawley; ANOVA, analysis of variance; IP, intraperitoneal; ICV, intracerebroventricular; IV, intravenous; PG, propylene glycol; PEG, polyethylene glycol.

in feeding regulation in Y1−/− mice. By using receptor selective antagonists proved in knockout mice, a clearer understanding of the role of the Y1 receptor will emerge.

In this study, we show that J-115814 is a potent and selective antagonist for the Y1 receptor in vitro and prove the actual specificity of J-115814 in Y1 deficient mice. Using this highly selective Y1 antagonist, we investigate the role of the Y1 receptor in physiological feeding regulation.

Experimental Procedures

Materials

NPY was purchased from Peptide Institute (Osaka, Japan). PYY and 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 Life Technologies (Grand Island, NY). All other chemicals were of analytical grade. J-115814 [(−)-2-[1-(3-chloro-5-isopropyloxycarbonylaminophenyl)ethylamino]-6-[2-(5-ethyl-4-methyl-1,3-thiazol-2-yl) ethyl]-4-morpholinopyridine] was synthesized by Banyu Pharmaceutical Co., Ltd. (Fig. 1).

Cell Culture. CHO-K1 cells expressing recombinant human Y1, Y2, and Y4 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 recombinant human Y5 receptors 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 and murine Y1 receptors and human embryonic kidney 293T cells expressing murine y6 receptors were grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS, penicillin-G (100 IU/ml), and streptomycin (100 μg/ml). All cells were grown in a 95% air/5% CO_2 humidified atmosphere at 37°C.

Binding Experiments

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 phenylmethylsulfonyl fluoride, 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) and ^{125}I-PP (25 pM), respectively. Bound and free peptides were separated by filtration using a GF/C glass filter (Whatman Japan, Tokyo, Japan) presoaked with 0.3% polyethylenimine. The remaining radioactivity on the filter was quantified using a TopCount (Packard Japan, Tokyo, Japan). Specific binding of ^{125}I-PYY and ^{125}I-PP was defined as the difference between total binding and nonspecific binding in the presence of 1 μM PYY and PP, respectively. Binding affinities are the average of more than three determinations.

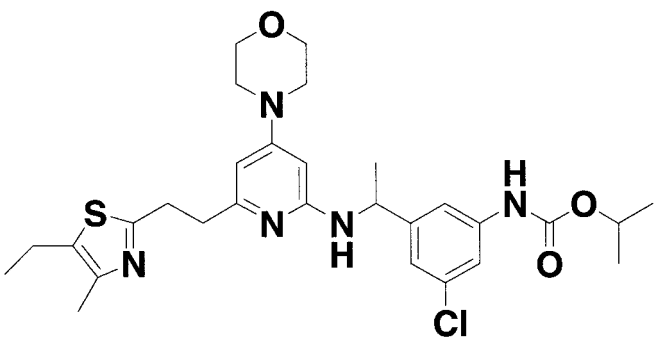

Fig. 1. Structure of J-115814.

Measurement of Intracellular Calcium Ion Concentrations

$[Ca^{2+}]_i$ was measured fluorometrically using a Ca^{2+}-sensitive fluorescent dye, Fura-2. 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. J-115814 or dimethyl sulfoxide was 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 were monitored with a CAF-110 intracellular ion analyzer (JASCO, Tokyo, Japan). $[Ca^{2+}]_i$ values were calculated according to the method reported previously (Grynkiewicz et al., 1985).

In Vivo Experimental Protocols

Male Sprague-Dawley (SD) rats (7–8 weeks) and male db/db mice (10–12 wks) were purchased from Charles River Japan (Tokyo, Japan). C57BL6 mice were from CLEA (Tokyo, Japan). Male Y1−/−, Y5−/−, and wild-type mice (10–12 weeks) were generated as reported previously (Kanatani et al., 2000b). They were housed in individual cages under controlled temperature (23 ± 2°C), humidity (55 ± 15%), and light/dark cycle (light, 7:00 AM–7:00 PM). Water and food pellets (CE-2; CLEA, Tokyo, Japan) were available ad libitum.

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.

Anorexigenic Effects of J-115814 in Rats. SD rats were anesthetized by IP injection of pentobarbital sodium (50 mg/kg; Dinabot, Osaka, Japan), and a 21-gauge guide cannula was implanted into the right lateral ventricle. The experiments were performed at least 1 week after surgery. The day before the experiment, food was changed to a palatable diet (protein, 15.9%; fat, 14.5%; carbohydrate, 57.0%; water, 6.6%; Oriental Bio Service, Tokyo, Japan) to guarantee satiety, and nocturnal food intake was measured. Rats that ate more than 15 g were used in the following experiments. In the case of intracerebroventricular (ICV) administration, each rat was injected with either NPY (5 μg, $n = 10$) or NPY + J-115814 (10, 30, or 100 μg, $n = 10$) dissolved in 50% propylene glycol (PG) with distilled water and their food intake was monitored for 2 h. The volume of ICV injection was 10 μl. With respect to intravenous (IV) administration, 0.3, 1, 3, or 10 mg/kg of J-115814 [dissolved in ethanol/polyethylene glycol (PEG) 400/saline (10:25:65)] or the respective vehicle was administered to groups of 10 animals 1 h before ICV administration of NPY dissolved in PBS. The injection was given between 9:00 AM and 11:30 AM. Consumption of a palatable diet, which was employed to maintain sufficient food intake, for 2 h after ICV NPY dosing was measured.

Anorexigenic Effects of J-115814 in db/db and C57BL6 Mice. Each group of 6 to 7 obese db/db and lean C57BL6 mice was IP administered 3, 10, or 30 mg/kg of J-115814 [dissolved in ethanol/PEG400/saline (10:25:65)] or the respective vehicle around the last hour of the light period. Nocturnal food consumption (14 h) was measured.

Anorexigenic Effects of J-115814 in Wild-Type, Y1−/−, and Y5−/− Mice. Mice were anesthetized with sodium pentobarbital (80 mg/kg IP). A permanent 24-gauge stainless steel cannula was stereotaxically implanted into the right lateral ventricle. Animals were allowed 1 week of recovery, and they were handled daily with mock injection to avoid nonspecific stress. Each mouse (n = 8–12/group)

was ICV-injected with NPY (5 μg) dissolved in PBS 1 h after IP administration of J-115814 (30 mg/kg), and food intake of a palatable diet was monitored for 2 h.

Plasma and Hypothalamic Concentrations of J-115814. Male db/db mice (n = 3) that had been surgically cannulated at the abdominal aorta and vein via the carotid artery and femoral vein, respectively, received an IP dose of 30 mg/kg (dissolved in 50% PG). Blood samples (0.05 ml) were drawn from the orbital venous plexus at selected time points after dosing. After a terminal blood collection and euthanasia, the brain was removed and the hypothalamic area was dissected.

Concentrations of J-115814 in plasma were determined after protein precipitation with 3 volumes of ethanol. Brain samples were homogenized with 2 and 0.5 ml of water, respectively. An aliquot of homogenates was deproteinized with 3 volumes of ethanol. Quantification was achieved by liquid chromatography/mass spectrometry/mass spectrometry (PerkinElmer SCIEX, Norwalk, CT).

Results

Selectivity and Potency of J-115814 in NPY Receptors. ^{125}I-PYY specific binding to human, rat, and murine Y_1 receptors in the cell membranes was inhibited by J-115814 with high affinities (K_i = 1.4, 1.8, and 1.9 nM) (Table 1). In contrast, J-115814 showed low affinity for other cloned NPY receptors such as Y_2, Y_4, Y_5, and Y_6 receptors (Table 1).

J-115814 dose dependently inhibited the NPY (10 nM)-induced $[Ca^{2+}]_i$ increase with an IC_{50} value of 6.8 nM in CHO cells expressing human Y1 receptors (Table 1), whereas J-115814 did not induce a $[Ca^{2+}]_i$ increase, even at 1 μM (data not shown).

Inhibitory Effects of J-115814 on NPY-Induced Feeding in Satiated SD Rats. ICV injection of NPY (5 μg) induced rapid and robust feeding in satiated SD rats (Fig. 2). J-115814 (100 μg alone) did not change the cumulative food intake compared with the respective vehicles (data not shown). Additionally, we did not observe any remarkable changes in gross behavior at any of tested doses of J-115814 and the respective vehicles alone (data not shown). Simultaneous ICV injection (10–100 μg) of J-115814 significantly suppressed food consumption induced by ICV NPY (5 μg) (Fig. 2A). In addition, IV administration of J-115814 (0.3 to 3 mg/kg) 1 h before ICV NPY (5 μg) also inhibited NPY-induced feeding with significant responses at 3 and 10 mg/kg (Fig. 2B).

Effects of J-115814 on Spontaneous Feeding in Obese db/db and Lean C57BL6 mice. Fig. 3 shows changes in spontaneous food intake in obese db/db mice and lean C57BL6 mice after IP injection of J-115814. J-115814 reduced spontaneous food intake with a minimum effective dose of 10 mg/kg in db/db mice. The amounts of spontaneous food intake in lean C57BL6 mice was considerably lower than that of db/db mice; however, J-115814 also attenuated the food intake in C57BL6 mice with the same minimum effective dose as in db/db mice. Residual food intake after the administration of 30 mg/kg of J-115814 was almost the same between db/db and C57BL6 mice. As shown in Fig. 4, 0.08 μM J-115814 was observed in the hypothalamus 15 h after administration. The concentration of J-115814 was more than 10-fold higher than the IC_{50} value of J-115814 in the calcium functional assays.

Anorexigenic Effects of J-115814 in Y1−/− Mice. ICV injection of NPY (5 μg) significantly stimulated feeding behavior in wild-type, Y1 deficient (Y1−/−), and Y5 deficient (Y5−/−) mice, although the level of food intake induced by ICV NPY was reduced considerably in Y1−/− mice compared with that of wild-type and Y5−/− mice (Fig. 5). IP-injected J-115814 (30 mg/kg) significantly suppressed food consumption induced by ICV NPY (5 μg) in wild-type and Y5−/− mice (Fig. 5, A and C), but had no effect on feeding in Y1−/− mice (Fig. 5B).

Discussion

Accumulating findings show that the Y1 receptor is involved in feeding regulation. However, the participation of multiple subtypes of NPY receptors including a possible novel subtype make it more difficult to correctly address the role of the Y1 receptor in ingestive behavior, especially in physiological feeding. In this study, we show that a highly selective Y1 antagonist, J-115814, which had no anorexigenic effects in Y1−/− mice, suppressed physiological feeding in lean and obese mice. Our results give a critical insight on the actual role of the Y1 receptor in feeding regulation.

J-115814 inhibited ^{125}I-PYY binding to human, rat, and mouse Y1 receptors with similar efficacy, but J-115814 showed low affinity for other NPY receptors in the membrane binding experiments. J-115814 potently inhibited the NPY-stimulated increase in intracellular calcium levels in CHO-K1 cells expressing human Y1 receptor. Moreover, we could not detect any significant cross reactivity with 50 other binding sites (data not shown). These results demonstrate that newly synthesized J-115814 is a potent and selective Y1 antagonist.

J-115814 considerably suppressed NPY-induced feeding after ICV and IV injection in satiated SD rats. Taken together with the in vitro profile, J-115814 suppresses NPY-induced food intake by inactivation of the Y1 receptor. To confirm the in vivo selectivity of J-115814, we compared the effects of J-115814 on NPY-induced food intake among wild-

TABLE 1

Pharmacological profiles of NPY and J-115814 for mouse NPY receptors

Y1, Y2, Y5, and Y6 affinities determined using ^{125}I-PYY, Y4 affinity determined using ^{125}I-PP. Data represent the mean of more than three independent determinations performed in duplicate.

	Binding affinity (K_i)							$[Ca^{2+}]_i$ Responses
	hY1	rY1	mY1	hY2	hY4	hY5	mY6	hY1
	nM							(EC_{50}; *nM*)
NPY	0.47 ± 0.04	0.98 ± 0.16	0.96 ± 0.21	0.10 ± 0.02	130 ± 37	1.2 ± 0.33	3.7 ± 0.88	0.76 ± 0.33
J-115814	1.4 ± 0.16	1.8 ± 0.18	1.9 ± 0.22	>10000	620 ± 50	6000 ± 460	>10000	(IC_{50}; *nM*) 6.8 ± 2.1

type, Y1−/− and Y5−/− mice. Although IP injected J-115814 significantly inhibited ICV NPY-induced feeding in wild-type and Y5−/− mice, J-115814 had no effect on feeding in Y1−/− mice. These results clearly demonstrate that J-115814 suppressed NPY-mediated feeding behavior by inhibiting the typical Y1 receptor, but not other subtypes of NPY receptors.

Maximum inhibition of J-115814 in ICV NPY-induced feeding was approximately 50% in satiated SD rats. The extent of feeding suppression was well matched to that in wild-type and Y5−/− mice. Moreover, it was also coincident with the reduced food intake evoked by ICV NPY in Y1−/− mice. These findings suggest that almost half of the food intake evoked by exogenous NPY is stimulated by the action of the Y1 receptor in rodents. Therefore, the Y1 receptor is one of the major feeding receptors in NPY-induced feeding.

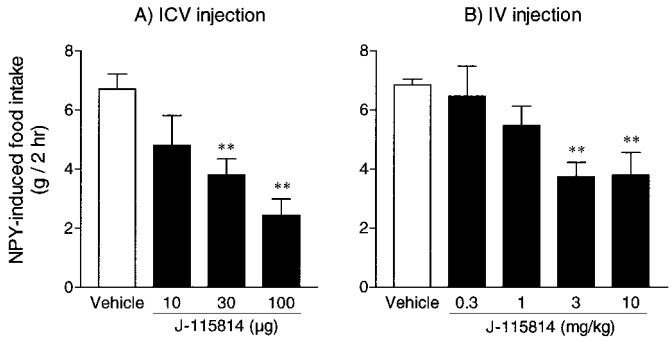

Fig. 2. Anorexigenic effect of J-115814 on NPY-induced feeding after ICV and IV injection in Sprague-Dawley rats. Both NPY (5 μg) and J-115814 were ICV-injected simultaneously. J-115814 was injected 1 h before ICV injection of NPY (5 μg). Data are expressed as the mean ± SE. $n = 10$; $^{**}P < 0.01$ (ANOVA followed by Bonferroni test).

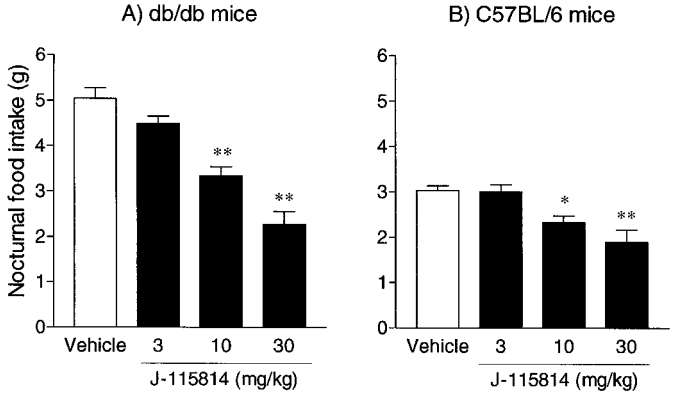

Fig. 3. Anorexigenic effect of J-115814 on spontaneous feeding in db/db and C57BL6 mice. J-115814 (3 to 30 mg/kg) was IP administered in both types of mice at the beginning of the dark cycle. Data are expressed as the mean ± SE. $n = 6$ to 7; $^{*}P < 0.05$, $^{**}P < 0.01$ (ANOVA followed by Bonferroni test).

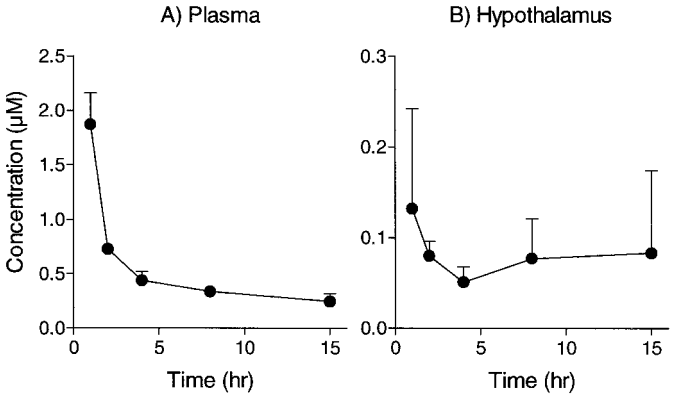

Fig. 4. Plasma and hypothalamic concentrations of J-115814 in db/db mice. J-115814 (30 mg/kg) was IP administered. Data are expressed as the mean ± SE. $n = 3$.

The residual food intake after J-115814 treatment also raises a question as to which NPY receptor subtypes are involved in residual food intake. The Y5 receptor has been proposed to be a feeding receptor based on the correlation between the in vitro functional and binding activity of different peptide agonists and their potent stimulation of food intake in rodent models (Gerald et al., 1996). This proposal was supported by the finding that food intake stimulated by ICV Y5-preferring agonists decreases significantly in Y5−/− mice (Marsh et al., 1998; Kanatani et al., 2000b). However, we could not detect a significant reduction of NPY-induced food intake in Y5 −/− mice (Kanatani et al., 2000b; Fig. 5). Furthermore, an orally active Y5 antagonist, L-152,804, failed to significantly attenuate NPY-induced feeding (Kanatani et al., 2000a). These findings suggest that the participation of the Y5 receptor in NPY-induced feeding is marginal. Although we could not completely exclude the participation of the Y5 receptor in wild-type and Y1−/− mice, the decrease in residual food intake after J-115814 administration in Y5−/− mice strongly suggests the involvement of additional NPY receptors in feeding regulation. The contribution of the remaining subtypes of NPY receptors, Y2, Y4, and Y6, in feeding regulation might not be very large (Gerald et al., 1996; O'Shea et al., 1997; Mullins et al., 2000). Thus, our findings support the concept that another novel NPY receptor is involved in NPY-mediated feeding regulation.

Increased hypothalamic expression of NPY and its mRNA had been reported in leptin-signaling deficient rodents such as Zucker fatty rats, db/db mice, and ob/ob mice and is considered to be an important cause of obesity (Sanacora et al., 1990; McKibbin et al., 1991; Dryden et al., 1995; Stephens et al., 1995). We previously demonstrated that Zucker fatty rats are sensitive to a peptide Y1 antagonist, 1229U91, compared with lean rats (Ishihara et al., 1998). Therefore, we evaluated the effects of J-115814 on food intake in db/db mice to compare it with that in lean control C57BL6 mice. IP administration of J-115814 (10 and 30 mg/kg) reduced spontaneous food intake with similar efficacy in both db/db and C57BL6 mice. In addition, food intake after the highest dose of J-115814 (30 mg/kg) in both types of mice was almost iden-

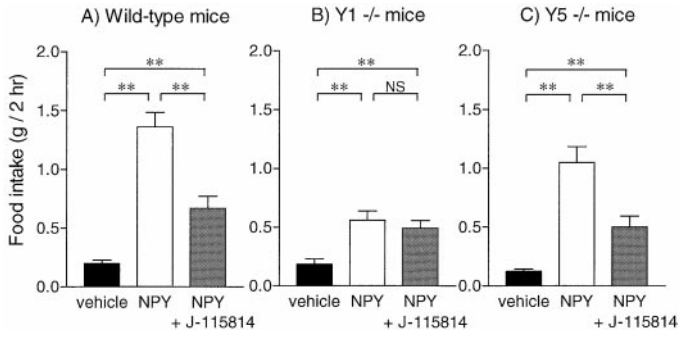

Fig. 5. Anorexigenic effect of J-115814 on NPY-induced feeding in wild-type, Y1−/− and Y5−/− mice. J-115814 (30 mg/kg) was IP injected 1 h before ICV injection of NPY (5 μg). Data are expressed as the mean ± SE. $n = 8$ to 12; $^{**}P < 0.01$ (ANOVA followed by Bonferroni test).

tical, but the amount of spontaneous food intake in db/db mice was 1.7-fold greater than that in C57BL6 mice. These findings showed that the Y1 receptor is also involved in spontaneous feeding and that the increased extent of Y1 participation might play a critical role, at least in part, in pathophysiological food intake in db/db mice. It has been reported that NPY-deficient ob/ob mice show a significant reduction of food intake and body weight compared with ob/ob mice (Erickson et al., 1996). Taken together, NPY signaling transduced by the Y1 receptor might be a critical factor in obesity caused by the inactivation of leptin signals.

Newly developed J-115814 is a potent and selective Y_1 receptor antagonist. The results of this study with Y1−/− mice clearly suggest that the anorexigenic effects of J-115814 are mediated by inactivation of the typical Y1 receptor. Consequently, the reduction of spontaneous food intake after J-115814 administration unquestionably suggests a pivotal role for the Y1 receptor in feeding regulation, and likely pathophysiological food consumption.

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References

Blomqvist AG and Herzog H (1997) Y-receptor subtypes—how many more? *Trends Neurol Sci* **20:**294–298.

Clark JT, Kalra PS, Crowley WR and Kalra SP (1984) Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology* **115:**427–429.

Daniels AJ, Matthews JE, Slepetis RJ, Jansen M, Viveros OH, Tadepalli A, Harrington W, Heyer D, Landavazo A, Leban JJ and Spaltenstein A (1995) High-affinity neuropeptide Y receptor antagonists. *Proc Natl Acad Sci USA* **92:**9067–9071.

Dryden S, Pickavance L, Frankish HM and Williams G (1995) Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats. *Brain Res* **690:**185–188.

Erickson JC, Hollopeter G and Palmiter RD (1996) Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science (Wash DC)* **274:**1704–1707.

Gerald C, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA and Weinshank RL (1996) A receptor subtype involved in neuropeptide-Y-induced food intake. *Nature (Lond)* **382:**168–171.

Grynkiewicz G, Poeni M and Tsien RY (1985) A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J Biol Chem* **260:**3440–3450.

Guan X-M, Yu H and Van der Ploeg LHT (1998) Evidence of altered hypothalamic pro-opiomelanocortin/neuropeptide Y mRNA expression in tubby mice. *Mol Brain Res* **59:**273–279.

Hipskind PA, Lobb KL, Nixon JA, Britton TC, Bruns RF, Catlow J, Dieckman-McGinty DK, Gackenheimer SL, Gitter BD, Iyengar S, Schober DA, Simmons RMA, Swanson S, Zarrinmayeh H, Zimmerman DM and Gehlert DR (1997) Potent and selective 1,2,3-trisubstituted indole NPY Y-1 antagonists. *J Med Chem* **40:**3712–3714.

Inui A (1999) Neuropeptide Y feeding receptors: Are multiple subtypes involved? *Trends Pharmacol Sci* **20:**43–46.

Ishihara A, Tanaka T, Kanatani A, Fukami T, Ihara M and Fukuroda T (1998) A potent neuropeptide Y antagonist, 1229U91, suppressed spontaneous food intake in Zucker fatty rats. *Am J Physiol* **43:**R1500–R1504.

Kalra SP, Dube MG, Sahu A, Phelps CP and Kalra PS (1991) Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc Natl Acad Sci USA* **88:**10931–10935.

Kanatani A, Ishihara A, Asahi S, Tanaka T, Ozaki S and Ihara M (1996) Potent neuropeptide Y Y1 receptor antagonist, 1229U91: Blockade of neuropeptide Y-induced and physiological food intake. *Endocrinology* **137:**3177–3182.

Kanatani A, Ishihara A, Iwaasa H, Nakamura K, Okamoto O, Hidaka M, Ito J, Fukuroda T, MacNeil DJ Van der Ploeg LHT, Ishii Y, Okabe T, Fukami T and Ihara M (2000a) L-152,804 orally-active and selective neuropeptide Y Y5 receptor antagonist. *Biochem Biophys Res Commun* **272:**169–173.

Kanatani A, Ito J, Ishihara A, Iwaawa H, Fukuroda T, Fukami T, MacNeil DJ, Van der Ploeg LHT and Ihara M (1998) NPY-induced feeding involves the action of a Y1-like receptor in rodents. *Regul Pept* **75–76:**409–415.

Kanatani A, Kanno T, Ishihara A, Hata M, Sakuraba A, Tanaka T, Tsuchiya Y, Mase T, Fukuroda T, Fukami T and Ihara M (1999) The novel neuropeptide Y Y1 receptor antagonist J-104870: A potent feeding suppressant with oral bioavailability. *Biochem Biophys Res Commun* **268:**88–91.

Kanatani A, Mashiko S, Murai N, Sugimoto N, Ito J, Fukuroda T, Fukami T, Mroin N, MacNeil DJ, Van der Ploeg LHT, Saga Y, Nishimura S and Ihara M (2000b) Role of the Y1 receptor in the regulation of neuropeptide Y-mediated feeding: Comparison of wild-type, Y1 receptor deficient, and Y5 receptor deficient mice. *Endocrinology* **141:**1011–1016.

Kesterson RA, Huszar D, Lynch CA, Simerly RB and Cone RD (1997) Induction of neuropeptide Y gene expression in the dorsal medial hypothalamic nucleus in two models of the agouti obesity syndrome. *Mol Endocrinol* **11:**630–637.

Marsh DJ, Hollopeter G, Kafer KE and Palmiter RD (1998) Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat Med* **4:**718–721.

McKibbin PE, Cotton SJ, McMillan S, Holloway B, Mayers R, McCarthy HD and Williams G (1991) Altered neuropeptide Y concentrations in specific hypothalamic regions of obese (fa/fa) Zucker rats: Possible relationship to obesity and neuroendocrine disturbances. *Diabetes* **40:**1423–1429.

Mullins DE, Guzzi M, Xia L and Parker EM (2000) Pharmacological characterization of the cloned neuropeptide Y y6 receptor. *Eur J Pharmacol* **395:**87–93.

O'Shea D, Morgan DGA, Meeran K, Edwards CMB, Turton MD, Choi SJ, Heath MM, Gunn I, Taylor GM, Howard JK, Bloom CI, Small CJ, Haddo O, Ma JJ, Callinan W, Smith DM, Ghatei MA and Bloom SR (1997) Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. *Endocrinology* **138:**196–202.

Pedrazzini T, Seydoux J, Kunstner P, Aubert JF, Grouzmann E, Beermann F and Brunner HR (1998) Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat Med* **4:**722–726.

Sanacora G, Kershaw M, Finkelstein JA and White JD (1990) Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. *Endocrinology* **127:**730–737.

Stanley BG, Kyrkouli SE, Lampert S and Leibowitz SF (1986) Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. *Peptides* **7:**1189–1192.

Stanley BG and Leibowitz SF (1984) Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci* **35:**2635–2642.

Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, MacKellar W, Rosteck PR, Schoner B, Smith D, Tinsley FC, Zhang X-Y and Heiman M (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature (Lond)* **377:**530–532.

Tatemoto K and Mutt V (1980) Isolation of two novel candidate hormones using a chemical method for finding naturally occurring polypeptides. *Nature (Lond)* **285:**417–418.

Tatemoto K, Carlquist M and Mutt V (1982) Neuropeptide Y - a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature (Lond)* **296:**659–660.

Wieland HA, Engel W, Eberlein W, Rudolf K and Doods HN (1998) Subtype selectivity of the novel nonpeptide neuropeptide Y Y1 receptor antagonist BIBO 3304 and its effect on feeding in rodents. *Br J Pharmacol* **125:**549–555.

White JD, Olchovsky D, Kershaw M and Berelowitz M (1990) Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. *Endocrinology* **126:**765–772.

Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F and Jeanrenaud B (1993) Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. *Endocrinology* **133:**1753–1758.

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