A potent neuropeptide Y antagonist, 1229U91, suppressed spontaneous food intake in Zucker fatty rats

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Ishihara, A., T. Tanaka, A. Kanatani, T. Fukami, M. Ihara, and T. Fukuroda. A potent neuropeptide Y antagonist, 1229U91, suppressed spontaneous food intake in Zucker fatty rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1500–R1504, 1998.—Neuropeptide Y (NPY) is one of the most potent orexigenic substances known. 1229U91 was found to be a potent and selective NPY antagonist. To elucidate a physiological role of NPY in hyperphagia in obese animals, we studied the effect of 1229U91 on spontaneous food intake in obese and lean Zucker rats. The food intake of Zucker rats was suppressed by intracerebroventricular administration of 1229U91 more potently in obese than in lean animals without abnormal behavior (31.7 and 67.3% inhibition at doses of 10 and 30 µg, respectively, in Zucker fatty rats and 22.2% inhibition at 30 µg in lean rats). This compound markedly suppressed NPY-induced food intake at 30 µg but did not affect galanin-induced food intake, suggesting that the feeding suppression seen in Zucker fatty and lean rats is pharmacologically and behaviorally specific. These results suggest that NPY is involved in feeding behavior in Zucker fatty rats and that NPY contributes to feeding to a greater degree in Zucker fatty than in lean rats. The hyperphagia in Zucker fatty rats may be due to the abnormal overactivation of the NPYergic system.

MATERIALS AND METHODS

Male Zucker fatty (fa/fa) and lean (Fa/Fa) rats (20–24 wk, Charles River Japan) and male SD rats (7–8 wk, Charles River Japan) were used. They were housed in individual cages under controlled temperature (23 ± 2°C), humidity (55 ± 15%), and light-dark cycle (0700–1900). Water and pellet food (CE-2, CLEA Japan) were available ad libitum.

Experiment 1. Zucker fatty and lean rats were anesthetized with ketamine (60 mg/kg ip; Sankyo, Tokyo, Japan) and chlorpromazine (6 mg/kg ip; Wako Pure Chemical, Osaka, Japan) mixture, and a sterile permanent 24-gauge stainless steel guide cannula was implanted stereotaxically. At least 1 wk after surgery, each group of 8–10 rats was injected either with vehicle (artificial cerebrospinal fluid containing 0.01% BSA) or with 10 or 30 µg of 1229U91 (synthesized as described (15)). Preliminary data showed that these doses of 1229U91 suppress exogenous NPY (1.2 nmol)-induced food intake in SD rats by 63 and 78%, respectively, suggesting that the doses are appropriate for the experimental purpose. The volume of intracerebroventricular injection was 5 µl. The injection was made during the last hour of the light period, and spontaneous food and water intakes were measured at 2, 14, and 24 h after administration.

Experiment 2. SD rats were anesthetized with pentobarbital sodium (50 mg/kg ip, Dainabot), and a 21-gauge guide cannula was implanted into the right lateral ventricle. At least 1 wk after surgery, each rat was injected with either NPY (1.2 nmol, n = 10), NPY + 1229U91 (30 µg, n = 10), galanin (3 nmol, n = 6), or galanin + 1229U91 (n = 7) and

NEUROPEPTIDE Y (NPY), a 36-amino acid neuropeptide, is a well-known potent orexigenic messenger molecule (27). It is widely distributed in the brain and is in particularly high concentration in the hypothalamus (26). Central administration of NPY increases food intake in rats, mice, pigs, and sheep (20–22, 27). NPY content and its mRNA level in the brain respond to ingesting status, such as food deprivation and refeeding (3, 24). Furthermore, centrally injected anti-NPY antiserum has been reported to suppress spontaneous food intake in Sprague-Dawley (SD) rats (11). From these data, NPY is thought to be one of the neurotransmitters that centrally regulate feeding behavior.

The genetically obese Zucker fatty rat is extensively used for the study of obesity. Hyperphagia, hyperinsulinemia, and decreased energetic efficiency are characteristics of Zucker fatty rats (6). Leptin resistance due to mutation of the leptin receptor is reported in this animal model (7). The primary cause of the Zucker fatty syndrome has not yet been clearly identified; however, a large dysregulation of hypothalamic neuropeptides, such as NPY, could be responsible for these abnormalities (1, 2). Levels of NPY (19) and its mRNA (25) and NPY secretion (10) are increased in the hypothalamic nuclei involved in the regulation of feeding behavior. NPY receptors are downregulated (18). Hyperphagia, obesity, and metabolic changes such as hyperinsulinemia, hypercorticosteroidemia, and hyperlipidemia, which were seen in Zucker fatty rats, were mimicked by repeated administration of NPY in normal rats (28). However, there is no direct evidence to indicate the relationship of NPY and hyperphagia of Zucker fatty rats because of the lack of a potent NPY antagonist.

1229U91 ([Ile-Glu-Pro-Dpr-Tyr-Leu-Arg-Tyr-NH2]6, cyclic (2,4′),(2,4′)-diamide, which was newly named GW1229 in a recent report of Bitran et al. (5)], was found to be a potent and selective NPY antagonist (8). This compound strongly suppressed the NPY- and fast-induced food intake (15). In the present study, we investigated the effect of 1229U91 to clarify the physiological role of NPY in spontaneous food intake in Zucker lean and fatty rats.

feeding behavior; galanin; obesity
their food intake was monitored for 2 h. The dosage of galanin selected was one that induced almost the same magnitude of food intake as 5 µg NPY. The volume of intracerebroventricular injection was 10 µl. The injection was done between 0900 and 1130.

All experimental procedures followed the Japanese Pharmacological Society Guideline for Animal Use. Results were given as means ± SE. Statistical analysis was made using repeated-measures ANOVA (expt 1) and ANOVA (expt 2) followed by Bonferroni test.

RESULTS

Experiment 1. Two-hour, 14-h (nocturnal), and 24-h food and water intakes in Zucker fatty rats are shown in Fig. 1. In Zucker fatty rats, the vehicle-injected group consumed 6.1, 19.3, and 28.1 g of food during 2, 14, and 24 h after injection, respectively. The total amount of feeding was not statistically different from that in the preadministration period. Intracerebroventricular administration of 1229U91 (10 and 30 µg) markedly suppressed the food intake in a dose-dependent manner (Fig. 1A). Water intake was also suppressed by 1229U91 (Fig. 1B). In Zucker lean rats, on the other hand, 2-, 14-, and 24-h food intakes of vehicle-treated group were 3.2, 13.1, and 17.7 g, respectively (Fig. 2). 1229U91 reduced food intake of the lean animals only at a higher dose (Fig. 2A). The suppression was slight but significant even at a 24-h cumulated index. Water intake did not differ significantly between each group (Fig. 2B). Other marked behavioral change such as change in motor activity, barrel rotation, catalepsy, or sedation was not observed after administration of this compound either in obese or lean rats.

Experiment 2. Figure 3 shows the effect of 1229U91 on extrinsic NPY- or galanin-induced food intake in SD rats. Intracerebroventricular injection of NPY (1.2 nmol) induced ~3 g of food intake. 1229U91, when coadministered with NPY, almost completely inhibited the NPY-induced food intake (Fig. 3A). Galanin also promoted significant food intake after intracerebroventricular administration. Simultaneously injected 1229U91 (30 µg) did not affect galanin-induced food intake (Fig. 3B).

DISCUSSION

The present results showed that intracerebroventricular injection of 1229U91 remarkably suppressed food intake in Zucker fatty rats. Ten and 30 µg of 1229U91 reduced spontaneous food intake in Zucker fatty rats for 24 h by 31.3 and 67.3%, respectively. 1229U91 is found to be a selective NPY receptor antagonist in vitro (8, 13, 15). Also in vivo, intracerebroventricular administration of this compound strongly suppressed NPY-induced food intake in satiated SD rats in a similar dose-dependent manner as in Zucker fatty rats (32.4, 63.0, and 77.7% with 3, 10, and 30 µg, respectively, unpublished observation). Together with these data, the present results strongly suggest that NPY is involved in the hyperphagia that is observed in Zucker fatty rats.
Because no abnormal change in general behavior was observed after administration of 1229U91, it is not likely that the suppression of food and water intake is due to abnormal behavioral change such as change in motor activity, catalepsy, or sedation. Furthermore, 1229U91 did not affect galanin-induced food intake at a dose at which this compound almost completely suppressed NPY-induced feeding. Thus the present results clearly show direct evidence that NPY is involved in feeding regulation, especially in Zucker fatty rats.

In the present experiment, 1229U91 also suppressed water intake in Zucker fatty rats. The effect, however, was significant only at higher dose. In Zucker lean rats, 1229U91 did not affect water intake at 30 µg, at which significant suppression in food intake was observed. The dissociation between suppression of food and water intake suggests that the reduction of water intake by 1229U91 is not probably due to the direct effect on drinking behavior. NPY is reported to induce water intake as well as food intake (27). However, the primary effect of NPY is stimulation of feeding, and food and water intake may change correlatively. In our preliminary experiment, 1229U91 did not affect drinking induced by water deprivation in SD rats (unpublished observation). Thus it is likely that 1229U91 primarily suppressed food intake rather than water intake.

Putative Y1 agonists [Pro34]NPY and [Leu31,Pro34]NPY elicit a strong feeding response, whereas putative Y2 agonists C9-NPY and NPY-(13—36) had a small or no effect at higher doses (14, 16, 29). These studies suggest that the Y1 receptor may be mainly involved in feeding behavior. Because we (15) and Hegde et al. (13) showed that 1229U91 is selective for Y1 over Y2 receptor, the present results suggest that the NPY may control feeding behavior via the Y1 receptor. However, the mediation of a variant of the Y1 subtype in feeding behavior has been suggested, because NPY-(2—36), which was less effective for Y1 receptor than NPY in other preparations (9, 12), is more effective than the intact molecule in eliciting feeding (29). Very recently, a novel NPY receptor, namely Y5, was reported and suggested to be involved in NPY-induced food intake (12). Because affinity of 1229U91 for Y5 receptor is not examined yet, the possibility that 1229U91 may exert the antiorexigenic effect via Y5 and/or other Y1-like receptor is not entirely neglected. Further investigation is necessary to clarify which subtype(s) of NPY receptor is involved in the antiorexigenic effect of 1229U91.

It is reported that an NPY antagonist, PYX-2, failed to decrease food intake in Zucker fatty rats (4), which is inconsistent with our present results. PYX-2 is reported to block NPY-induced carbohydrate feeding, but it does not modify the total energy intake (17). Thus in vivo efficacy and/or stability of PYX-2 may not be enough to suppress total energy intake. Alternatively, PYX-2 is reported to bind the NPY receptors in rat brain membrane, which are predominantly of the Y2 receptors. Only Y2 receptors bind to COOH-terminal fragments of NPY (32). These suggest that PYX-2, modified COOH-terminal fragments of NPY (31), may have a much stronger affinity for Y2 receptors than for Y1 receptors. This may be the reason why PYX-2 is ineffective, because the contribution of Y2 receptor in feeding control is rather small.

1229U91 slightly suppressed spontaneous food intake in Zucker lean rats, suggesting that NPY also regulates daily food intake in normal animals without overeating. However, the effect in lean rats was significantly weaker than that in fatty rats. There is indirect evidence that suggests the hypothesis that the NPYergic system is overactivated in Zucker fatty rats: NPY content and secretion along with the NPY mRNA level are increased in the hypothalamic nuclei, which are involved in the regulation of feeding behavior (10, 19, 25). The increased content, mRNA levels, and secretion of NPY suggest that the contribution of NPY in the regulation of physiological feeding is increased in Zucker fatty rats compared with in lean rats.

Despite the overactivation of NPY system, Zucker fatty rats are reported to be less sensitive to the exogenously administered NPY than Zucker lean rats (30). Not only does the high concentration of endogenous NPY overactivate the NPY receptors but it may also downregulate or mask the receptors, resulting in a decrease in the number of unoccupied receptors. The decrement may explain the lower sensitivity of fatty rats to exogenous NPY. Indeed, total hypothalamic receptor density was reduced in Zucker fatty compared with lean rats (18).
The overactivation of the NPY system may be because of impaired signaling of the hormonal product of the ob gene, leptin. Leptin resistance due to mutation of the leptin receptor is reported in Zucker fatty rats (7). Reduced hypothalamic leptin signaling may stimulate NPY production and release, which may contribute to the increased food intake and reduced energy expenditure (23).

Perspectives

The dramatic suppression of feeding in Zucker fatty rats observed in the present study indicates that NPY regulates physiological feeding behavior in this obese animal. It is well known that NPY inhibits energy expenditure in brown adipose tissue and enhances energy deposition in white adipose tissue. When it is chronically administered into a normal rat’s brain, NPY mimics hormonal and metabolic changes of obesity such as hyperinsulinemia and liver and adipose tissue lipogenic activity. Obesity arises from an imbalance between energy intake and expenditure, and the ideal antiobesity drug will need to address both aspects of the energy balance equation. The findings about NPY suggest that chronic treatment with NPY antagonists is expected not only to suppress physiological appetite but also to ameliorate obesity, and therefore NPY antagonists may be useful for the treatment of eating disorders and obesity.

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