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Co-Administration of Dopamine D_1 and D_2 Agonists Additively Decreases Daily Food Intake, Body Weight and Hypothalamic Neuropeptide Y Level in Rats

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Key Words

Dopamine agonists, food intake · Dopamine agonists, body weight change · Hypothalamic NPY · Co-administration of D_1 and D_2 agonists

Abstract

This study investigated whether co-administration of dopamine D_1 and D_2 agonists might additively inhibit the feeding effect and whether this effect was mediated by the action on hypothalamic neuropeptide Y (NPY). The D_1 agonist SKF 38393 (SKF) and D_2 agonists apomorphine (APO) or quinpirole (QNP) were administered, alone or in combination, to examine this possibility. In single administration, decreases of daily food intake were observed only in rats treated twice a day with a higher dose of SKF, APO or QNP. However, combined administration of D_1 and D_2 agonists, with each agent at a dose that alone did not induce anorexia in one daily treatment, exerted a significant effect. These results reveal that co-activation of D_1 and D_2 receptors can additively reduce daily food intake and body weight. The same treatment also decreased the level of hypothalamic NPY 24 h post-treatment. These results suggest an additive effect during combined activation of D_1 and D_2 receptor subtypes to decrease food intake and body weight that are mediated by the action of hypothalamic NPY. Similar to the effects seen in healthy rats, combined D_1/D_2 administration was also effective in the reduction of food intake in diabetic rats, revealing the efficiency of D_1/D_2 agonist in the improvement of hyperphasia in diabetic animals.

Experimental evidence indicates that various neurotransmitters play an important role in the regulation of feeding behavior [23]. Dopamine (DA) is one of these candidates [24]. However, the effect of DA on feeding behavior appears paradoxical. Several factors, such as the brain area (e.g. anorectic action in the perifornical hypothalamus and stimulatory action in the lateral hypothalamus) or DA dose (low doses enhanced feeding and higher doses inhibited it) have shown some connection to the response [15, 19]. Nevertheless, DA is generally accepted as an inhibitory agent in the control of feeding behavior [5, 9].

DA exerts its inhibitory effect via the DA receptors [28]. DA receptors have several subtypes (D_1, D_2, D_3, D_4 and D_5) [25], which are also widely noted as D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) subtypes on the basis of physiological, pharmacological and biochemical studies [11]. Previous studies indicated that both general [7] and specific D_1 and D_2 [4, 21] agonists could decrease food intake, however, it was unknown whether the combined administration of D_1 and D_2 agonists might elicit a synergistic or additive effect on the decrease of intake. Based on the observation that in certain instances maxi-

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mal responses of central D_2 agonists should accompany endogenous DA to activate D_1 receptor sites [8, 10], we believed that the combined administration of D_1 and D_2 agonists in rats should exert a significant effect on feeding.

In the present study, chemical agents related to DA were used mainly to characterize the difference between receptor subtypes of D_1-like and D_2-like receptors. In addition, DA agonists used in this study, including D_1 agonist SKF 38393 (SKF) and D_2 agonists apomorphine (APO) and quinpirole (QNP), are agents that have been proved to have central inhibitory action on food intake with systemic administration [23, 28].

Neuropeptide Y (NPY), which is known as an orexigenic agent, is widely distributed in the central nervous system with high concentrations in the hypothalamus [16, 26]. Hypothalamic NPYergic neurons projecting from the arcuate nuclei to the paraventricular nuclei and the perifornical area are postulated to control the feeding behavior in rodent animals [6]. Therefore, we secondarily examined whether the combined activation of DA receptor subtypes might affect the protein level of hypothalamic NPY. By using histochemical and pharmacological analysis, an interaction between the dopaminergic system and the hypothalamic NPYergic system was proved [14], revealing the possible causative relationship between activation of D_1 or D_2 receptors and alteration in hypothalamic NPY level.

Methods

Animals

Male rats of the Wistar strain, weighing 200–300 g, were housed individually in a cage, maintained at 22 ± 2°C in a room with a 12-hour light-dark cycle (light on at 06:00 a.m.), and habituated to frequent handling. Water and chow were freely available throughout the experiment. The administration of drugs and food intake checks were performed at the beginning of the dark cycle (06:00 p.m.). The measuring of food intake and body weight change was calculated with respect to the weight of the previous day. All rats in all treatments were drug-naive when tested.

Drugs

Apomorphine hydrochloride was purchased from Sigma (St. Louis, Mo., USA), SKF 38393 [(±)-1-phenyl-2,3,4,5,-tetrahydro(1H)-3-benzazepine-7,8-diol] from Research Biochemicals Inc. (Natrick, Mass., USA), quinpirole from Eli Lilly & Co. (USA) and NPY kits from Peninsula Labs Inc. (Belmont, Calif., USA). SKF and QNP were dissolved in physiological saline. APO was dissolved in 0.9% saline containing 0.2% ascorbic acid to prevent oxidation. All drugs were freshly dissolved in solution to a final volume of 1 mg/ml.

Single Administration with a D_1 or D_2 Agonist

To determine the effects of DA agonists on daily food intake and body weight change, rats were treated twice a day with SKF, APO or QNP. Using doses previously found effective in feeding studies [1], rats were treated with SKF (1 and 4 mg/kg, i.p.), APO (1 and 4 mg/kg, i.p.) or QNP (0.05 and 0.2 mg/kg, i.p.) twice a day at the beginning of the dark cycle (06:00 p.m.) and at midnight (12:00 p.m.) to induce an effective suppression of daily food intake.

Combined Administration of D_1 and D_2 Agonists

SKF (1 or 4 mg/kg, i.p.) was co-administered with either APO (1 and 4 mg/kg, i.p.) or QNP (0.05 and 0.2 mg/kg, i.p.). The dose ratios for the combined administration of SKF/APO were 1/1, 1/4, 4/1 and 4/4. The dose ratios for SKF/QNP were 1/0.05, 1/0.2, 4/0.05 and 4/0.2. The measuring of food intake and body weight change was determined at 24 h after drug administration.

Hypothalamic NPY Determination

The DA agonist-treated and vehicle-treated (including pair-fed and normal-fed) rats were killed by decapitation at 24 h after drug or vehicle administration. Their hypothalamus was removed immediately and placed respectively in 2 *N* acetic acid and sonicated for 20 s at 4°C. After sonication, one part (50 µl) of the tissue pellets was dissolved in 1 *N* NaOH and assayed for protein content [17]. The other part was immediately boiled for 5 min, and then cooled on ice and centrifuged for 20 min at 3,000 rpm. The supernatant was stored at −20°C until assay. Each sample was then measured by the radioimmunoassay (RIA) specific for each NPY as described previously [2]. Authentic antibody against NPY and radioactive tracer ^{125}I-labeled NPY were purchased from Peninsula Labs Inc. The amount of food in pair-fed animals was equal to that of the APO- or QNP-treated animals (approx. 20 ± 1 g/day) in the single administration experiment, and was equal to that of the SKF/APO- or SKF/QNP-treated animals (approx. 17 ± 2 g/day) in the combined administration experiment.

Effects of Combined Administration of D_1 and D_2 Agonists on Diabetic Rats

To induce a state of diabetes, rats were given a single injection of streptozotocin (STZ, 65 mg/kg, i.v.) in sterile saline into the femoral vein under pentobarbital anesthesia (40 mg/kg, i.p.) after 3 days' fasting. A fasting blood glucose level >350 mg/dl 72 h after STZ injection confirmed diabetes. Hyperglycemia was sustained for at least 3 weeks and was confirmed every 3 days during this stage. Clinical features of the disease (polyuria, polydipsia, polyphasia, weight loss and malaise) were also observed during this stage. Blood glucose levels were measured using the glucose oxidase method (Max Planck Institute, Wiesbaden, Germany). One week after establishing basic hyperglycemia, animals were used for further study.

Co-administration of SKF with either APO or QNP was applied in diabetic rats for 4 days. The dose ratios for the combined administration of SKF/APO were 1/1 and 4/4, and for SKF/QNP 1/0.05 and 4/0.2. The measuring of food intake was determined at 24 h after the drug administration.

Statistics

Statistical data were assessed by one-way ANOVA followed by post-hoc Dunnett's test. Values are expressed as mean ± SEM for each group of animals.

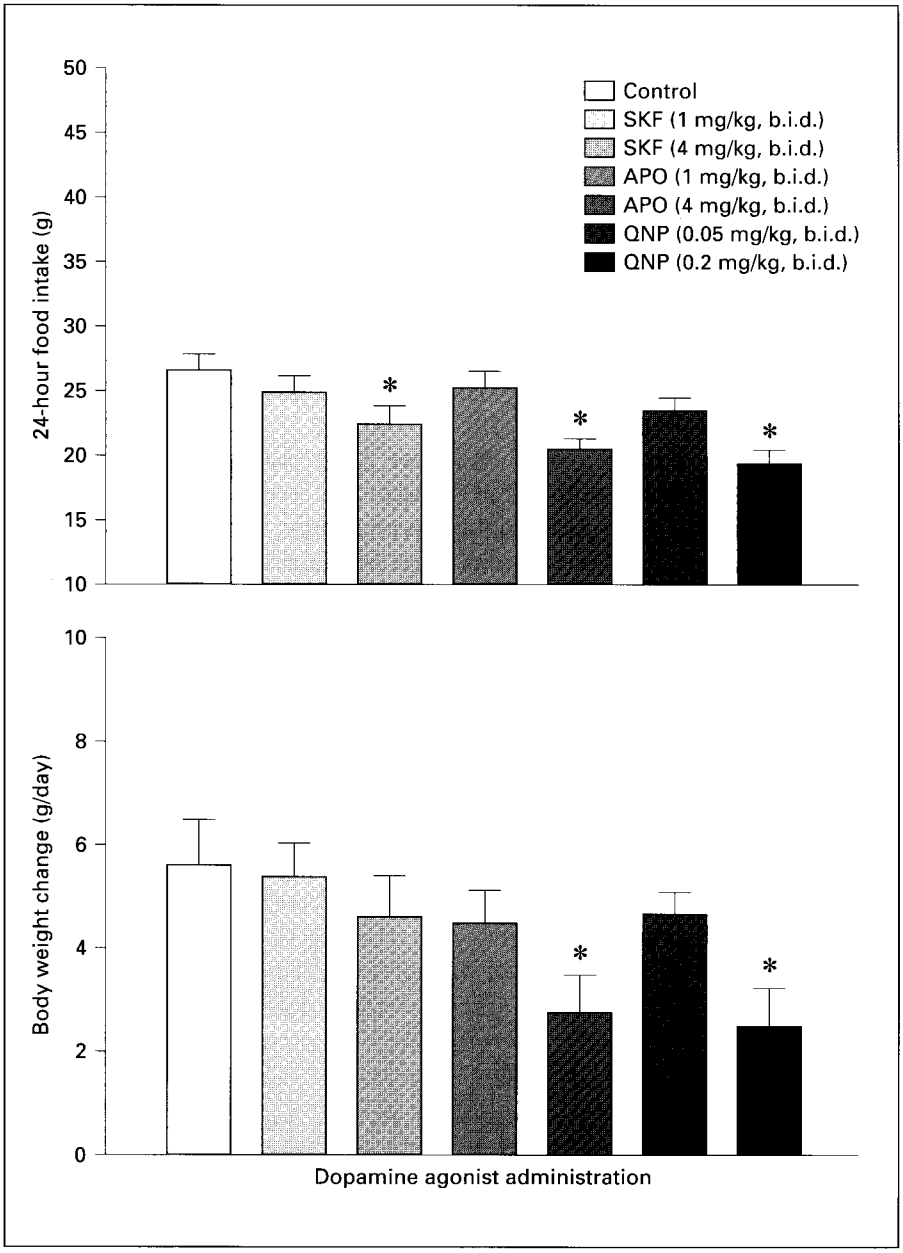

Fig. 1. Effects of single administration of dopamine D_1 or D_2 agonist on 24-hour food intake (upper panel) and body weight change (lower panel). Decreases of food intake and body weight were observed only in rats treated twice a day with a higher dose of D_1 or D_2 agonist. SKF: SKF 38393; APO: apomorphine; QNP: quinpirole; b.i.d.: twice a day. Data are means ± SEM, n = 8 per group. * $p < 0.05$ vs. controls (Dunnett's test).

Results

Figure 1 reveals the effect of single administration with SKF, APO or QNP on daily food intake (upper panel) and body weight change (lower panel) when injected twice a day. The results indicated that treatment with APO at a higher dose (4 mg/kg, i.p., b.i.d.) or QNP at a higher dose (0.2 mg/kg, i.p., b.i.d.) both significantly decreased the daily intake and body weight change, but treatment with SKF at a higher dose (4 mg/kg, i.p., b.i.d.) decreased daily food intake only ($p < 0.05$, Dunnett's test). Statistical analysis with ANOVA showed a significant effect on food intake: $F(6,49) = 5.1$, $p = 0.0004$ and body weight: $F(6,49) = 3.01$, $p = 0.0138$. However, when injected once a day, none of the single administrations of DA agonists at similar dose patterns could induce anorexia (data not shown).

Figure 2 (left panel) illustrates the effect of co-administration with SKF/APO on daily food intake. Results indicated that SKF/APO at dose ratios of 1/1, 1/4, 4/1 or

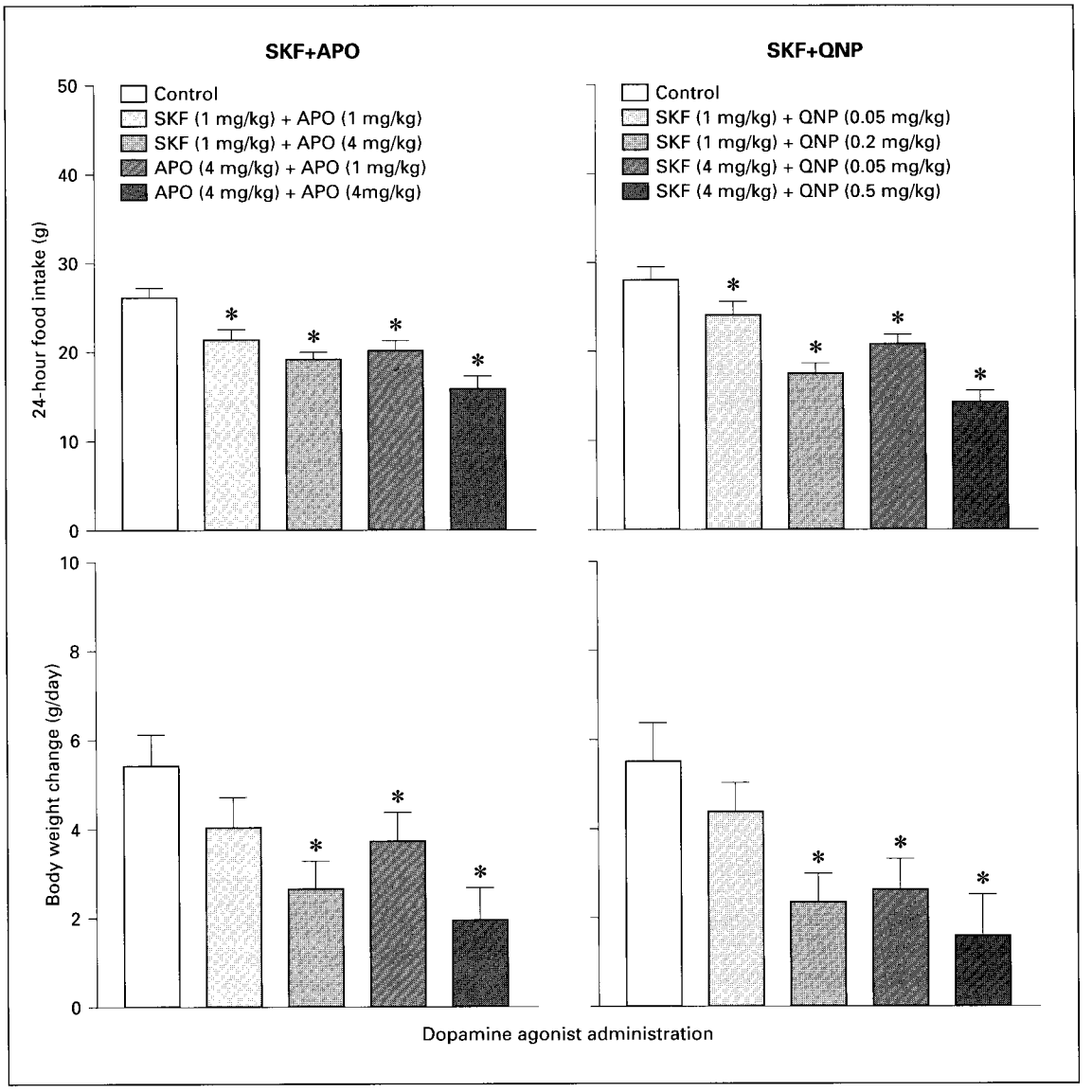

Fig. 2. Effects of combined administration with dopamine D_1 and D_2 agonists on daily food intake (upper panel) and body weight change (lower panel). Combined administration of D_1 and D_2 agonists, each agent at a dose that would not by itself reduce food intake with once daily treatment, produced a significant decrease in daily food intake and body weight change. SKF: SKF 38393; APO: apomorphine; QNP: quinpirole. Data are means ± SEM, n = 8 per group. * $p < 0.05$ vs. controls (Dunnett's test).

4/4 mg/kg could induce an inhibitory effect on 24-hour intake [$F(4,35) = 11.1$, $p < 0.0001$] and that dose ratios of 1/4, 4/1 or 4/4 mg/kg could induce a significant body weight change [$F(4,35) = 3.64$, $p = 0.0139$]. Figure 2 (right panel) depicts the effects of SKF/QNP. The results indicated that the combined administration of SKF/QNP at the dose ratios of 1/0.05, 1/0.2, 4/0.05 and 4/0.2 mg/kg could induce an inhibitory effect on 24-hour intake [$F(4,35) = 19.1$, $p < 0.0001$] and that at dose ratios of 1/0.2, 4/0.05 or 4/0.2 mg/kg could induce a significant body weight change [$F(4,35) = 4.28$, $p = 0.0064$].

Table 1 indicates the effects of D_1 and/or D_2 agonists on the protein level of hypothalamic NPY. The results indicated that, when injected twice a day, single administration with APO at a higher dose (4 mg/kg) or QNP at a higher dose (0.2 mg/kg) could decrease the protein level of

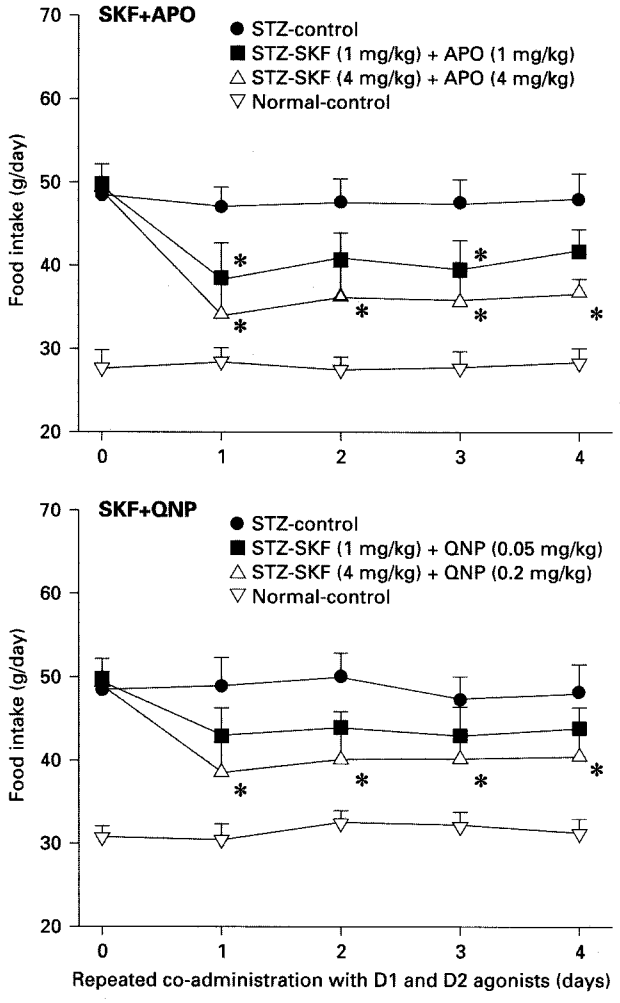

Fig. 3. Effects of repeated co-administration of dopamine D_1 and D_2 agonists on daily food intake for 4 days in streptozotocin (STZ)-induced diabetic rats. Combined administration of SKF with either APO (upper panel) or QNP (lower panel), both induced an anti-hyperphagic effect on daily food intake. SKF: SKF 38393; APO: apomorphine; QNP: quinpirole. Data are means ± SEM, n = 7–8 per group. * $p < 0.05$ vs. STZ-control group for each treatment day (Dunnett's test).

Table 1. Effects of D_1 and/or D_2 agonists' administration on the protein level of hypothalamic NPY; the protein level of NPY was measured by RIA at 24 h after intraperitoneal injection of drugs

Drug administration (n = 8 each group)	Protein level of hypothalamic NPY, pg/ml
Normal-fed control	66.67 ± 3.38
Single administration	
Pair-fed control	67.97 ± 2.55
SKF 38393	
1 mg/kg, i.p., twice a day	65.75 ± 2.47
4 mg/kg, i.p., twice a day	61.36 ± 2.91
Apomorphine	
1 mg/kg, i.p., twice a day	62.84 ± 2.58
4 mg/kg, i.p., twice a day	57.10 ± 2.20*
Quinpirole	
0.05 mg/kg, i.p., twice a day	62.49 ± 2.62
0.2 mg/kg, i.p., twice a day	54.36 ± 1.86*
Combined administration	
Pair-fed control	65.74 ± 2.72
SKF 38393+Apomorphine	
1 mg/kg/day + 1 mg/kg/day	57.74 ± 2.15*
1 mg/kg/day + 4 mg/kg/day	51.88 ± 1.64*
4 mg/kg/day + 1 mg/kg/day	53.12 ± 2.35*
4 mg/kg/day + 4 mg/kg/day	49.88 ± 2.99*
SKF 38393+Quinpirole	
1 mg/kg/day + 0.05 mg/kg/day	55.12 ± 2.12*
1 mg/kg/day + 0.2 mg/kg/day	49.12 ± 1.98*
4 mg/kg/day + 0.05 mg/kg/day	53.01 ± 2.11*
4 mg/kg/day + 0.2 mg/kg/day	48.75 ± 2.38*

The amount of food in pair-fed animals was equal to that of the D_2 agonist-treated animals in the single administration study, and was equal to that of the D_1/D_2-treated animals in the combined administration study.

* $p < 0.05$ vs. pair-fed controls (Dunnett's test).

hypothalamic NPY, but single administration with SKF at both doses (1 and 4 mg/kg) failed to produce this effect [$F(6,49) = 2.68$, $p = 0.0249$]. In combined administration, the results revealed that, when injected once a day, co-administration of SKF/APO at dose ratios of 1/1, 1/4, 4/1 and 4/4 mg/kg or SKF/QNP at dose ratios of 1/0.05, 1/0.2, 4/0.05 and 4/0.2 mg/kg, could all dose-dependently decrease the protein level of hypothalamic NPY [$F(8,71) = 5.36$, $p < 0.0001$].

Figure 3 (upper panel) illustrates the effect of repeated co-administration of SKF/APO on daily food intake in STZ-diabetic rats during a 4-day period. Results indicated that SKF/APO at dose ratios of 4/4 and 4/1 mg/kg could induce an inhibitory effect on daily intake ($p < 0.05$, Dunnett's test). Figure 3 (lower panel) depicts the effect of SKF/QNP. The results indicated that repeated co-administration with SKF/QNP at a dose ratio of 4/0.2 mg/kg, but not 1/0.05 mg/kg, could induce an inhibitory effect on daily intake ($p < 0.05$, Dunnett's test). These results revealed the efficiency of combined D_1 and D_2 administration in the reduction of daily food intake in diabetic rats.

Discussion

In the single administration study, we found that daily double injections with a higher dose of D_1 or D_2 agonist were necessary for rats to decrease their 24-hour pattern of food intake.

Compared with single administration, combined administration of D_1 and D_2 agonists, each agent being in a dose that scarcely affected feeding behavior by itself if treated once a day, induced a significant decrease in food intake and body weight. These results revealed that co-administration of D_1/D_2 agonists could additively decrease the effect on feeding. This additive effect might be produced due to a simultaneous co-activation of D_1 and D_2 receptors. There are several behavioral examples of receptor cooperation of D_1 and D_2 receptors. For example, the combined administration of SKF and QNP has an additive effect in inducing oral stereotyped behavior in rats [3], and the development and expression of behavioral sensitization of psychomotor stimulants require an integrated activation of D_1 and D_2 receptors [20]. Therefore, it is possible that the combined activation of D_1 and D_2 receptors may exert an additive action on the decrease of 24-hour feeding.

Similar results were also observed in the change of hypothalamic NPY level. Combined administration of D_1 and D_2 agonists, each being in a dose that did not by itself affect NPY content, produced a significant effect on the decrease of NPY content. Also, NPY content decreased more in combined D_1/D_2 administration than in single administration. These results revealed that co-activation of D_1 and D_2 receptor subtypes could additively decrease NPY content. There are several reports indicating an interaction between the dopaminergic system and hypothalamic NPYergic system in the regulation of rodent feeding [14, 15]. Thus, D_1 and D_2 agonists in combination might act together to reduce NPY content and in turn to decrease daily food intake and body weight.

We ruled out the possibility that the changes in NPY level were simply secondary to reduced feeding, rather than the direct action of DA agonists on hypothalamic NPY, because pair-fed (non-drug-treated) animals showed no change in NPY level compared with that in normal-fed animals.

It was noteworthy that single administration of SKF was less efficient than that of APO or QNP in decreasing daily food intake, body weight change and NPY content. In SKF-treated rats, daily food intake showed less reduction but body weight and NPY levels remained unchanged when compared with those in APO- or QNP-treated rats. These might not be produced due to receptor binding affinity. The affinity of SKF at D_1 receptors is slightly stronger than those of APO and QNP at D_2 receptors [18]. A possible reason might be due to the receptor's co-activation induced by APO and QNP treatments. The D_2 agonists APO and QNP are also regarded as mixed D_1/D_2 and D_2/D_3 agonists, respectively [12], and therefore may co-activate the related DA receptor subtypes simultaneously to decrease food intake, as conditioned in combined administration. However, this possibility needs further investigation.

In addition to normal rats, our data revealed that co-application of D_1 and D_2 agonists in diabetic rats that show hyperphagia and elevated hypothalamic NPY [22] can also reduce the food intake during a 4-day period, indicating the efficiency of combined D_1/D_2 administration in the improvement of hyperphagia in diseased animals. The metabolic and neuroendocrine responses to dopamine D_1/D_2 agonists' treatment are very similar to those of leptin [8]. The hormone leptin is synthesized by adipose tissue and plays a crucial role in the regulation of food intake and energy expenditure [29]. Treatment with both DA agonists and leptin can attenuate hyperphagia, obesity and elevated hypothalamic NPY [13, 27]. Thus, it is rational to predict that combined administration of D_1 and D_2 agonists may contribute to lower the protein level of NPY in the drug-induced improvement of hyperphagia.

In conclusion, the present study suggests that combined administration of D_1 and D_2 agonists may additively decrease daily food intake, body weight and hypothalamic NPY level.

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