Expression of dopaminergic receptors in the hypothalamus of lean and obese Zucker rats and food intake

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Received 12 February 2002; accepted in final form 29 May 2002

Fetissov, Serguei O., Michael M. Meguid, Tomoi Sato, and Li-Hua Zhang. Expression of dopaminergic receptors in the hypothalamus of lean and obese Zucker rats and food intake. Am J Physiol Regul Integr Comp Physiol 283: R905–R910, 2002; 10.1152/ajpregu.00092.2002.—As revealed by previous microdialysis studies, basal and food intake-accompanied dopamine release significantly differs in the hypothalamus of obese vs. lean Zucker rats. In the present study, we determined whether dopaminergic receptors are also compromised in obesity. Dopaminergic D1 and D2 receptor mRNA expression was studied in the ventromedial hypothalamus (VMH), lateral hypothalamic area (LHA), and the adenohypophysis (AH) of obese and lean Zucker rats using RT-PCR technique. In obese Zucker rats, we found an upregulation of D1 receptor mRNA in the VMH and AH and a downregulation in the LHA, whereas D2 receptor mRNA was downregulated in both the VMH and LHA, but not changed in the AH, compared with lean rats. Also, an increase of D1 receptor staining was seen in the paraventricular nucleus of obese rats by immunohistochemistry. We selected the VMH to test if the observed changes in the dopamine receptor expression of obese rats induce behavioral sensitization to dopamine as expressed by hyperphagia. The overnight food-deprived rats received a single VMH injection (10 nmol) of sulpiride (D2 receptor antagonist) or saline as control, then food was provided and 1-h food intake was measured. Food intake after sulpiride vs. saline injection was decreased in the LHA, whereas D2 receptor mRNA was downregulated in both the VMH and LHA, but not changed in the AH, compared with lean rats. Also, an increase of D1 receptor staining was seen in the paraventricular nucleus of obese rats by immunohistochemistry. We selected the VMH to test if the observed changes in the dopamine receptor expression of obese rats induce behavioral sensitization to dopamine as expressed by hyperphagia. The overnight food-deprived rats received a single VMH injection (10 nmol) of sulpiride (D2 receptor antagonist) or saline as control, then food was provided and 1-h food intake was measured. Food intake after sulpiride vs. saline injection was greater in obese rats but was not different in lean rats. Our data suggest that downregulation of D2 receptor in the hypothalamus at least in the VMH induces behavior sensitization for having large meals. Low D2 receptor expression may be causal for an exaggerated dopamine release observed in obese rats during food ingestion and for reduced satiety feedback effect of dopamine. High level of D1 receptor expression in the VMH and low in the LHA may also contribute to the specific feeding pattern in obese rats represented by large meal size and low meal number.

 monoamine; meal size

THE ORIGIN OF OBESITY in the Zucker rat is a missense mutation in the gene coding for the leptin receptor (31, 39). This diminishes the sensitivity for leptin signaling to the brain (43) leading to numerous adaptive changes of the central regulatory systems, which develop into and characterize the Zucker phenotype, manifested by hyperphagia, positive energy balance, and obesity.

The ventromedial hypothalamus (VMH), the lateral hypothalamic area (LHA), and several other hypothalamic nuclei are well established as the centers for the regulation of metabolism (for review, see Ref. 34) and have potent modulatory influence on daily food intake (FI) mediated mainly via lower brain stem nuclei (35). Daily FI is a function of meal size (MZ) and meal number (MN) [FI = MZ × MN], which constitutes a feeding pattern (24). The rat on ad libitum diet is able to perfectly adjust its MN in accordance to its MZ to maintain constancy of daily FI, suggesting an existence of neurochemical mechanisms responsible for sensing and measuring both, MZ and postmeal interval. The obese Zucker rat is not an exception to this rule and displays a feeding pattern characteristic for obesity that is characterized by large MZs and low MN.

The observations that experimental manipulation in the hypothalamus, which would induce changes in FI, also led to a change in feeding pattern (9, 22) provide evidence that changes in MZ and frequency occur favorably and in concordance with the desired metabolic effect. For instance, for an efficient control of body weight and stimulation of energy expenditure, it is advisable to increase MN and reduce MZ (17). Thus, by understanding the mechanisms of induction of large MZs, it would be possible to find an alternative therapeutic strategy for the treatment of obesity.

Because normal function of dopaminergic neurotransmission has been shown to be indispensable for feeding and survival (38), it is now clear that any abnormality involving the dopaminergic system will be reflected by changes in feeding behavior. By using in vivo microdialysis, we showed that in the normal rat, the release of dopamine (DA) in the LHA and VMH correlates with MZ and postmeal intervals, reflecting the MN (25, 26). Although in the obese Zucker rats, food ingestion is accompanied by an exaggerated release of DA in these two brain areas (28, 44). Thus, in the obese Zucker rat, FI-accompanied hypothalamic release of DA is different from that in the lean Zucker.

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rat, indicating abnormal dopaminergic neurotransmission.

DA acts on its specific receptors belonging to the G protein-coupled receptor family, which are classified into two subgroups: D1-like and D2-like, according to their function to stimulate or to inhibit adenylyl cyclase, respectively (for review, see Ref. 27). Normal function of dopaminergic signaling is closely dependent on the level of expression of dopaminergic receptors (for review, see Ref. 11). Thus, in the present work, we measured the expression of DA D1 and D2 receptor mRNA in the hypothalamus of obese and lean Zucker rats and tested our hypothesis that differences in the levels of receptor expression are relevant to hyperphagia of the obese rat.

MATERIALS AND METHODS

Subjects. The experiments were approved by the Committee for the Humane Use of Animals, State University of New York, Upstate Medical University at Syracuse. The animal care provided was in accordance with guidelines established by the National Institutes of Health. Obese (fa/fa) and lean (Fa/Fa) male Zucker rats 2.5–3 mo old were purchased from Harlan (Indianapolis, IN). Rats were housed in holding wire mesh cages for 1 wk after purchase to acclimate them to the constant study environmental conditions: 12-h light cycle (0600–1800), 26 ± 1°C room temperature, and 45% humidity. They were fed a standard rat chow (Diet 5008;Ralston Purina, St. Louis, MO). Food and tap water were available ad libitum.

RNA isolation and RT-PCR. Rats (n = 12) were killed by decapitation at 1000–1100. Fresh tissues of the VMH and LHA were collected using Micron Punch (Harvard Apparatus, Holliston, MA). The adenohypophyses were also harvested after removing the neural lobe. RNA was isolated using an established technique, previously described in detail (33). Relative quantitative RT-PCR was performed using primers, standard curves, and specificity controls as described in the companion paper by Sato et al. (33).

Surgery. Obese and lean Zucker rats (n = 16) were anesthetized with ketamine, xylazine, and acepromazine (150: 30:5 mg/ml) 0.6 ml/kg im and mounted in a stereotaxic apparatus (Kopf Instrument, Tujunga, CA) with an incisor bar –3.3 mm below the interaural line. Stainless steel cannula with a 22-gauge guide (Plastics One, Roanoke, VA) was implanted unilaterally into the medial hypothalamic area, anterior VMH:AP –2.0 mm, 0.5 mm lateral from the midline, and 8.0 mm ventral from the dura mater (37). The cannula was fixed on the skull using stainless steel screws and acrylic dental cement. At the end of the experiments, the correct position of the guide was verified by routine histology.

FI and sulpiride administration. Before the operation for cannula placement, the rats were acclimated to the Automated Computerized Rat Eater Meter (ACREM) cages (23). Their daily FI and each MZ and MN were measured for 1 wk. After the operation, the rats were replaced once more into their individual ACREM cages until the characteristic feeding pattern (see Fig. 2) was reestablished in all rats. At 10 AM the next day after an overnight food deprivation, obese and lean rats were divided into two groups (each n = 8) in a crossover randomized design. The first group of rats received a single VMH injection of 10 nmol/0.5 μl of sulpiride, a DA D2 receptor antagonist (Sigma Chemicals, St. Louis, MO), via the implanted cannula, whereas the second group serving as control received a single injection of 0.5 μl of saline, adjusted to a comparable pH 5.0, via the implanted cannula. Then food was provided and 1-h FI was measured. Three days after the first injection, the experiment was repeated. But this time the first group of rats was injected with saline, whereas the second group received sulpiride, which was dissolved in saline with 2 N acetic acid with a final pH 5.0 (29).

Immunohistochemistry. Immunohistochemistry was performed as previously described in detail (7). Briefly, obese and lean rats (n = 6) were anesthetized with ketamine, xylazine, and acepromazine (150:30:5 mg/ml) 0.8 ml/kg im and perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde. The brains were rapidly dissected and kept for 4 h in the same fixative, then 50-μm sections were cut on a vibratome in phosphate-buffered saline, pH 7.4. The primary antibodies for D1 receptor (1:100), the secondary biotinylated antibodies, the avidin-biotin-peroxidase complex, the peroxidase diaminobenzidine substrate, and the blocking peptides were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Sections were studied under a light microscope.

Data analysis. Quantitation of the PCR product was performed on the agarose gel using SigmaGel 1.0 software (SPSS Science, Chicago, IL.). D2 receptor mRNA expression was analyzed by measuring the total intensity of its two isoforms (long and short). Data are expressed as the percentage of change relative to β-actin and analyzed using unpaired Student’s t-test. FI and feeding pattern data were analyzed using unpaired Student’s t-test and are expressed as means ± SE.

RESULTS

Dopaminergic D1 and D2 receptor mRNA expression. As measured relative to the level of expression of β-actin mRNA, which expression was not significantly different between obese and lean rats (Fig. 1), in the VMH, obese vs. lean rats displayed a higher level of D1 (49.9 ± 11.5 vs. 13.4 ± 4.8%, respectively; P < 0.02) and a lower level of D2 (0.3 ± 0.07 vs. 5.8 ± 0.9%, respectively; P < 0.001) receptor mRNA expression. In the LHA, the levels of D1 and D2 mRNA were found to be lower in the obese vs. lean rats (D1: 3.2 ± 2.6 vs. 25.3 ± 5.6%, P < 0.01; and D2: 1.01 ± 0.2 vs. 10.9 ± 1.9%, P < 0.001, respectively). In the adenohypophysis, the level of D1 receptor was higher in the obese vs. lean rats (92 ± 19 vs. 19 ± 6%, respectively, P < 0.01), whereas the level of D2 was not significantly different between the obese and lean rats (35 ± 4.8 and 42 ± 6.8%, respectively).

FI and feeding pattern. A characteristic feeding pattern was observed in lean and obese rats during their basal conditions (Fig. 2). Obese rats displayed hyperphagia, large MZ, and low MN. FI (means ± SE) during 1 h after sulpiride vs. saline injection was greater in the obese rats (7.1 ± 0.6 vs. 5.7 ± 0.2 g, respectively; P < 0.03). It did not change significantly in the lean rats 5.0 ± 0.4 vs. 5.2 ± 0.6 g, respectively (Fig. 3).

Immunohistochemistry. Immunohistochemical detection of D1 receptors revealed an increased intensity of staining in the medial hypothalamus in obese vs. lean rats, which was particularly evident in the magnocellular neurosecretory neurons (Fig. 4). Application of the primary antibodies preabsorbed by their block-
ing peptides 10^{-6} \text{ M} \text{ overnight on the sections showed no immunostaining.}

**DISCUSSION**

The hypothalamus contains the incertohypothalamic (A13) and tuberohypophysial (A12) dopaminergic neurons (6). Therefore, the D_2 receptor mRNA detected in the LHA and VMH may, in part, reflect pre- and postsynaptic D_2 receptors. Regarding postsynaptic localization of D_1 and D_2 receptors in the hypothalamus, D_1 receptor immunoreactivity is present in the arcuate and periventricular nuclei (15), in the magnocellular and parvocellular paraventricular nucleus and the zona incerta (5), whereas D_2 receptor occurs in the arcuate, supraoptic, suprachiasmatic, and mammillary nuclei (16).

A considerable amount of data exists regarding the regulation of both D_1 and D_2 receptors in the basal ganglia, showing that chronic depletion of DA upregulates both D_1 and D_2 receptors (for review, see Ref. 11). Thus, if dopaminergic receptor expression in the hypothalamus is controlled by DA release, then by analogy to the basal ganglia, it is possible that an upregulation of D_1 receptor mRNA in the VMH and a decrease in the LHA of obese rats could be due to a low or high local DA concentration, respectively. This hypothesis is supported by our previous microdialysis studies, where such differences in the baseline DA concentrations in the obese vs. lean Zucker rats were found (21, 44).

**Fig. 1.** D_1 and D_2 receptors and \( \beta \)-actin PCR products from the ventromedial hypothalamus (VMH) of obese and lean rats and the levels of expression of dopaminergic D_1 and D_2 receptor mRNA relative to \( \beta \)-actin (100\%) in the VMH, adenohypophysis, and lateral hypothalamic area (LHA) in obese and lean Zucker rats. Arrows indicate long and short isoforms of D_2 receptor.

**Fig. 2.** Characteristic food intake and feeding pattern in ad libitum-fed obese and lean Zucker rats (means ± SE; *\( P < 0.001 \)). Obese rats compensate for their huge meal sizes by reducing their daily meal number, whereas lean rats that have smaller meal sizes display more frequent number of meals per day.

**Fig. 3.** Food intake (means ± SE) measured for 1 h after a single injection of 10 nmol of sulpiride or saline into the medial hypothalamus of overnight food-deprived obese and lean Zucker rats.
Consequently, if hyperdopaminergia induced by deletion of the DA transporter (DAT) was a cause for a decrease in D2 receptor (12), one explanation for the downregulation of D2 receptor in both the LHA and VMH could be an exaggerated release of DA concomitantly with FI (28, 44). An upregulation of DAT in the hypothalamus of obese Zucker rats was found by Figlewicz et al. (10), suggesting that the DAT may partly compensate for a low presynaptic D2 receptor. An alternative explanation could be that the expression of D2 receptor is modulated by a nondopaminergic mechanism. This may involve, for instance, a direct interaction with leptin signaling, because leptin has been shown to inhibit DA release from the nerve terminals (2).

The condition of a chronic change in the level of receptor expression is a prerequisite of behavioral sensitization. Direct evidence for the behavioral sensitization by the level of dopaminergic receptor expression in the obese rats was found using sulpiride, a selective D2 receptor antagonist. Sulpiride was injected into the VMH of obese and lean rats, but a hyperphagic response was found only in obese rats. Thus by aggravating the already low level of D2 receptor, it was possible to increase FI, an effect that suggests that in obese rats a low level of D2 receptor may contribute to the pathogenesis of large meals. The fact that lean rats did not respond to sulpiride injection further suggests that under normal conditions, blocking of D2 receptor may be compensated by other dopaminergic receptors such as D1 receptor, whereas in obesity this mechanism is altered. Data that D1 and D2 receptors synergize to inhibit feeding (32) and a mix of D1/D2 DA receptor agonists are needed to alleviate obesity (1) support the idea that expression of both D1 and D2 dopaminergic receptors is involved in the pathogenesis of hyperphagia during obesity.

Thus, the present data may, in part, explain why more DA in the VMH of obese rats is required for meal termination, because of the low postsynaptic D2 expression, where DA acts as a satiety signal via a feedback to the hindbrain feeding centers. Accordingly, an impaired satiety effect via neuropeptide Y (NPY) (30) and peripheral hormones such as pancreatic peptide amylin (20). Also, a direct effect of DA in the VMH to regulate adiposity is possible, because its neurons contribute to the autonomic pathways that innervate white fat tissue (36). At the presynaptic level, a relatively low D2 receptor expression may trigger an exaggerated DA release via insufficient autoreceptor function. D2 receptor in the VMH may also provide feedback to the tuberoinfundibular dopaminergic neurons (18) and therefore may modulate the endocrine metabolic component of FI via regulation of hypophysial hormones.

Regarding D1 receptor, DA release is synchronized between the VMH and LHA (8), therefore a high level of D1 receptor in the VMH and a low level in the LHA of the obese rats may simultaneously accentuate and diminish, respectively, the effect of DA in these two areas. Because DA induces feeding and MZ in the medial hypothalamic nuclei while inhibiting them in the LHA (9, 13, 19, 45), the changes of D1 receptor found in obese rats should result in an elevated DA stimulation of MZ. Accordingly, an increase of D1 immunostaining found in the paraventricular nucleus of obese rats supports the idea that medial hypothalamic D1 receptor is associated with an increase in MZ.

An interesting comparison can be drawn between the dopaminergic receptor status in the VMH of obese rats and in the brain of chronic drug abusers (42). In both, there are conditions of low basal DA concentrations, periodical exaggerated DA release associated either with food or drug intake, and a decrease in D2 receptor with an increase of D1 receptor expression. A number of addictive behaviors has been found to be associated with low expression or dysfunctional D2 receptor (for review, see Ref. 3). Obesity can be added to this list because an association between D2 receptor mutation and obesity was reported (4). The availability of D2 receptor is also significantly reduced in the striatum of obese humans (41) as well as rats (14). Thus, a downregulation of D2 receptor may represent a common pathogenic mechanism contributing to obesity and to a number of addiction syndromes linked to behavior, which would facilitate DA release into the brain areas “craving” for DA.

In conclusion, we found that obese Zucker rats, which display a feeding pattern consisting of large MZ and small MN, have an altered level of dopaminergic receptor mRNA expression in the hypothalamus. A low level of D2 receptor expression in the ventromedial hypothalamus was found to be relevant to hyperphagia. We postulate that changes in dopaminergic recep-
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	or expression in the medial and lateral hypothalamus induce behavior associated with large meals, which, in turn, are favorable for the development of obesity.

Perspectives

Because the dopaminergic system is critically involved in feeding behavior, further research on the regulation of DA receptor expression with particular attention to D2 receptor is needed. More detailed data on the neurochemical phenotype of the hypothalamic neurons expressing different subtypes of dopaminergic receptors are also needed to understand the mechanism of the dopaminergic involvement in the control of FI. A perspective direction of research may include the interaction between the leptin receptor and D2 receptor as well as an effect of other hormones, including insulin, NPY, and ghrelin and other brain messengers on the neurochemical phenotype of the hypothalamic neurons. More detailed data involved in feeding behavior, further research on the mechanism that improves the function of D2 and other dopaminergic receptors, it would be possible to normalize the dopaminergic neurotransmission in the brain with the ultimate purpose of treating additive behavior, including the hyperphagia of obesity.

We thank Dr. C. Förster, Dept. Med. Nutrition, Karolinska Institutet, Sweden for critical reading of the manuscript.

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REFERENCES