Altered basal and stimulated accumbens dopamine release in obese OLETF rats as a function of age and diabetic status

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Anderzhanova E, Covasa M, Hajnal A. Altered basal and stimulated accumbens dopamine release in obese OLETF rats as a function of age and diabetic status. Am J Physiol Regul Integr Comp Physiol 293: R603–R611, 2007. First published June 6, 2007; doi:10.1152/ajpregu.00301.2007.—The Otsuka Long-Evans Tokushima Fatty (OLETF) rat lacking the CCK-1 receptor is hyperphagic, prefers palatable and high-calorie meals, and gradually develops obesity and type 2 diabetes. To determine dopamine levels in this strain, we used in vivo quantitative (no net flux) microdialysis at three different ages representing nondiabetic (8 wk), prediabetic (18 wk), and diabetic (56 wk) stages in OLETF and age-matched lean Long-Evans Tokushima Otsuka (LETO) controls. Results showed significantly elevated basal dopamine levels in the caudomedial nucleus accumbens of OLETF rats compared with LETO at younger ages (8 wk: 20.10 ± 5.61 nM vs. 15.85 ± 5.63 nM; 18 wk: 7.37 ± 3.71 nM vs. 4.75 ± 1.25 nM, means ± SD). In contrast, at 56 wk of age, a profound decline in extracellular dopamine concentrations was seen in both strains with a tendency for a greater effect in OLETF rats (1.78 ± 0.40 nM vs. 2.39 ± 0.42 nM). Further, extracellular fraction, an index for reuptake, was higher in 56-wk-old OLETF compared with LETO (0.648 ± 0.049 vs. 0.526 ± 0.057). Potassium-stimulated dopamine efflux revealed an increased capacity of vesicular pool in OLETF rats compared with LETO across all age groups with an accentuated strain difference at 56 wk. These findings demonstrate altered striatal dopamine functions (i.e., increased stimulated release and uptake) in obese OLETF rat. This could be due to the lack of functional CCK-1 receptors, or metabolic and hormonal factors associated with the development of obesity and insulin resistance, or both.

Obesity: CCK-1 receptor; type 2 diabetes; overeating; no-net-flux microdialysis

Obesity is a global pandemic that continues to accelerate and increases the risk of multiple medical conditions (14). In addition to genetic and environmental influences (25, 35, 69), food-related factors, such as palatability and energy content, also play important role in development of obesity (21, 57, 64, 76). Among other integrative neural substrates, dopamine (DA) has been suggested to be involved in various aspects of eating, including intake, food selection, satiety, and energy expenditure (5, 43, 46, 66, 72, 77). Although much is unclear about DA’s specific role(s) in inducing chronic overeating on high-calorie meals as a plausible antecedent of obesity, there is strong evidence showing that dopaminergic neurotransmission is altered in obese subjects. For example, lower D2 receptor density was found in the striatum of both obese humans and animals (31, 38, 39, 73). Conversely, antipsychotic drugs that block DA D2 receptors increase appetite and weight gain (1).

Furthermore, chronic high-fat diet-induced obesity leads to decreased DA turnover in the hypothalamus (45). Additionally, the availability of dopamine transporter (DAT) that regulates synaptic DA concentration by reuptake of the transmitter into presynaptic terminals is higher in mice that are prone compared with those resistant to chronic high-fat diet-induced obesity (39). Collectively, these findings suggest that DA signaling is reduced in obese subjects. In contrast, numerous studies have suggested that obese rats actually have higher hypothalamic DA levels than lean rats (for a review, see Ref. 46). A study by Huang et al. (38) found higher D2 receptor and tyrosine hydroxylase mRNA expression in the core of the nucleus accumbens (NAC), and ventral tegmental area (VTA), in obesity-prone compared with obesity-resistant mice, suggesting that an increased DA signaling may increase susceptibility to overeating and development of obesity. Consistent with this notion, it has been shown that DA is not only required for maintenance of higher body weight in leptin-deficient (ob/ob) mice (67) but also is sufficient to restore feeding in cachectic DA knockout mice (66). The contradictory findings in the literature may be explained by the different dietary regimens and obesity models used, as well as whether the role of DA in either the energy regulatory or reward circuities was assessed. Nonetheless, because alterations to the synaptic machinery may also reflect a compensatory regulation to altered DA release downstream from obesity and associated metabolic factors, one must be cautious when interpreting relationships between DA functions and obesity based on indirect indices (i.e., receptor and transporter expression). An alternative solution to resolve this intricacy is to measure the actual extrasynaptic DA levels with respect to basal and stimulated release over the course of development of obesity and its metabolic associates.

To achieve this goal, in the present study, we used the Otsuka Long-Evans Tokushima fatty (OLETF) rat an animal model that gradually develops obesity and type 2 diabetes (42, 49). The OLETF rat has a congenital CCK-1 receptor deficiency, resulting from a 6,847-base pair deletion spanning the promoter region and the first and second exons of the CCK-1 receptor gene (68). CCK-1 receptors are the receptor subtype that mediate CCK’s actions in satiety (48). Consistent with this role, OLETF rats have deficits in the control of meal size. The size of spontaneous meals is almost double that of the LETO controls. Furthermore, OLETF rats have deficits in responding to CCK, and gastric and intestinal preload (16, 49). In addition to diminished sensitivity to postingestive satiation signals,
there has been accumulating evidence demonstrating deficits in central CCK signaling in this strain (6). Recently, we demonstrated that, OLETF rats express increased real and sham intake of normally preferred sucrose solutions (19) and an increased generalized avidity to sweet tastants (28). Thus, similar to humans susceptible to dietary obesity, OLETF rats share multiple characteristics: increased meal-size, reduced satiation, defenseless metabolic response to high-calorie diets, and an increased sensitivity to food reward (i.e., overeating driven by palatability).

In addition to its use as a model for diet-induced obesity and diabetes, the OLETF rats offer an opportunity to study CCK modulation of DA. This is based on anatomical and functional evidence demonstrating interactions at multiple levels between the mesencephalic DA and CCK systems (32, 36, 40, 62). Of particular importance, a recent microdialysis study, using net-flux method demonstrated higher cocaine- and amphetamine-induced DA release in the dorsal but not the ventral striatum in OLETF rats compared with LETO rats (23). However, conventional (i.e., net-flux) microdialysis does not provide quantitative measurement of extracellular DA concentration, such as tonic DA efflux, actual release, as well as quantitative estimates of reuptake (52). Because these variables are relevant to the behavioral phenotype under physiological conditions (i.e., when not challenged pharmacologically with DA mimetics), in the present study, we used the no-net-flux method of microdialysis. This modified technique provides an estimate of the extracellular neurotransmitter concentration, and a measure of the extraction fraction of the microdialysis probe with respect to time (7).

By controlling for probe recovery, this latter parameter can also be used to monitor DA uptake, a functional measure of DAT activity (10, 50, 51). On the basis of these theoretical considerations and recent studies demonstrating validity of the method (8, 10, 12, 13), we aimed at measuring basal and potassium-stimulated DA release and estimating uptake in OLETF rats compared with age-matched, lean LETO controls. To investigate the relationship between DA and obesity and to control for the effect of escalating insulin resistance, we repeated these assays in separate subsets of rats at three different ages representing nondiabetic, prediabetic, and diabetic stages (11, 19, 28).

**MATERIALS AND METHODS**

**Subjects.** Male OLETF (n = 17) and LETO (n = 16) rats were obtained as a generous gift of the Tokushima Research Institute, Otsuka Pharmaceutical, Tokushima, Japan. Separate sets of rats of both strains at ~8 wk (5 LETO and 7 OLETF), ~18 wk (6 LETO and 6 OLETF) and ~56 wk of age (5 LETO and 4 OLETF) (all within ±1 wk) were used in the experiments. All rats were housed individually in mesh-floored, stainless-steel hanging cages and maintained in a temperature-controlled vivarium on a constant 12:12-h light-dark cycle (lights on at 0700). Tap water and pelleted rat chow (Purina 5001) were available ad libitum throughout experiments. All protocols used were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by The Pennsylvania State University Institutional Animal Care and Use Committee. The authors further attest that all efforts were made to minimize the number of animals used and their suffering.

**Surgery and microdialysis.** On the day of experiment, rats were anesthetized with chloral hydrate (Sigma-Aldrich, St. Louis, MO), dissolved in physiological saline (loading dose 400 mg/kg ip; supplemented with 80 mg/kg ip every 2 h) and placed on a heating pad (37°C) (Stoelting, Wood Dale, IL). Each rat was fixed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and two microdialysis probes (CMA 12 Elite, 2-mm membrane length, 20 kDa; CMA Microdialysis AB, Solna, Sweden) were implanted into right and left NAC. The probe implants were targeted at the posterior medial extent of the accumbens shell/core region. Accordingly, the stereotaxic coordinates for the 8-wk age group were AP 1.2 mm (from the bregma), ML 1.1 mm, and DV −7.5 mm (from the skull). For 18- and 56-wk age groups were AP 1.3 mm, ML 1.1 mm, and DV −7.5 mm (54). Probes were kept continually perfused with artificial cerebrospinal fluid (aCSF; containing 145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl2, 1.0 mM MgCl2, 2.0 mM Na2HPO4, ascorbic acid 0.25 mM, set at pH = 7.4), at a flow rate of 1.0 µl/min. Before used, the aCSF solution was filtered through a 0.22-µm filter.

**Quantitative no-net-flux microdialysis.** Briefly, no-net-flux microdialysis assesses the ability of the target brain structure to supply endogenous DA to a microdialysis probe and remove exogenous DA applied by the probe. When the concentration of DA perfused through the probe is equal to the extracellular concentration, there will be no net diffusion of the DA molecule into or out of the probe. In practice, a mathematical approximation is used to get an extracellular concentration of neurotransmitter. This method allows performing a correct evaluation of actual content of DA without excessive overestimation or underestimation of its extraneuronal concentration.

Theoretical considerations, as well as practical observations of no-net-flux method show that extraction fraction index, the slope of a regression line, is a useful tool for nondirect dopamine uptake evaluation (10, 51). Thus, an angle between regression line and x-axis reflects an activity of DAT. As had been suggested, relatively small changes in extraction fraction index correspond to significant changes in uptake (9).

Accordingly, in our design, after a 90-min equilibration period, probes were perfused in a random order with aCSF containing DA in the following concentrations (DA influx, or DAin): 0, 1.0, 2.0, 4.0, and 8.0 µM, respectively) with 60 min of equilibration period for each concentration. During this period, samples were collected in 10-min intervals.

**Potassium-stimulated DA release.** After completion of no-net-flux measurements with the rat still under anesthesia, aCSF with no DA was perfused for 60 min to establish a stable baseline. Then, the normal aCSF was replaced by switching to a stream of a modified aCSF solution containing 100 mM KCl using a low dead-volume liquid switch (Univentor, Bulebal, Malta). The high-potassium aCSF solution was made to isosmotic to normal aCSF by modifying Na+ content (to 48.3 mM) and was perfused over a 20-min period, providing two equal samples within this interval. Following this period (“stimulation”), perfusion buffer was changed back to normal aCSF, and four consecutive 10-min samples were collected (“wash-out”). The internal capacity of the inlet tubing was 1.8 µm. In this arrangement, there was a 1.8-min delay between the onset of high-potassium aCSF infusion and its appearance in the outflow from the microdialysis probe.

**Dopamine and metabolite assays.** Once collected, all microdialysis samples were stored at ~80°C and analyzed together within 18 h following sampling. Dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined by reverse-phase HPLC coupled with coulometric detection. The chromatographic system consisted of a refrigerated autosampler (Model 542, ESA Biosciences, Chelmsford, MA), a solvent delivery isocratic system (Model 582, ESA Biosciences) and a coulometric detector (CoulArray, Model 5600A; ESA Biosciences). The mobile phase contained 0.09 M sodium phosphate, 0.05 M sodium phosphate, 1.7 mM octanesulfonic acid, 0.05 mM EDTA, and 10% acetonitrile (vol/vol) and adjusted to pH = 3.0. All reagents used for the mobile phase were analytical grade. The mobile phase was filtered through a 0.22-µm nylon filter and pumped through the system at a flow rate of 0.7 ml/min. Monoamines were separated on
an ESA column (C18, 150 mm × 3.2 mm, 3 μm). Guard cell was set at +350 mV, the working electrode potentials E1 and E2 were −150 and +200 mV, respectively. Injection volume was 8 μl and limit of detection (LOD) for DA was 0.019 pg/μl (or 0.1 nM). LOD for DOPAC was not examined since the acumbens microdialysate concentration for this metabolite is usually in the hundred-nanomole range. Retention time for DOPAC and DA were 2.8 and 3.3, respectively. The monoamine levels were quantified by external standard curve calibration using peak area for quantification.

**Oral glucose tolerance tests.** Oral glucose tolerance tests (OGTTs) were performed at 8, 20, and 52 wk in a different set of rats (n = 3–5) with similar body weights to rats used in microdialysis studies. The rationale for not performing OGTTs in the actual subjects was to reduce potential effects from food deprivation and procedural stress on the DA system. The test was administered following a 16-h fast, when an oral glucose load (2 g/kg) was delivered to each rat orally via latex gavage. Blood glucose was measured before and at 30, 60, 90, and 120 min postglucose loading using a standard glucose meter (LifeScan, One-Touch Basic, Malpitas, CA). Animals were classified as diabetic if the peak level of plasma glucose were ≥300 mg/dl (16.7 mmol/l) and a peak glucose level at 120 min >200 mg/dl (11.1 mmol/l) (42).

**Histology.** After completing the microdialysis tests, rats were perfused transcardially, brains were removed, and 50-μm cresent violet stained sections were verified under microscope for probe location using the Paxinos and Watson atlas (54).

**Data analysis.** All data are expressed as means ± SD. Statistical analyses were computed with Statistica software for PC (version 6.1.; StatSoft, Tulsa, OK). Body weight and OGTTs were calculated for each age group and compared between strains at each time point using Student’s t-tests. Neurochemical data from both hemispheres were collapsed for statistical analyses, since there was no statistical difference between samples obtained from the right and the left NAC in either strain at any time point.

All no-net-flux microdialysis data were expressed in nanomoles. The net change of DA [DA_{in} − DA_{out} (DA_{nout})], where DA_{in} and DA_{out} are the DA concentration in the input and output perfusates, respectively, was calculated for each animal and then averaged for each perfusion group. The average net change in DA was plotted respectively, was calculated for each animal and then averaged for each perfusion group. The average net change in DA was plotted

**RESULTS**

**Body weight.** As expected, body weight significantly increased with aging in both strain (F_{2,27} = 10.7, P < 0.001). However, post hoc tests revealed that OLETF were heavier than LETO at all ages (8 wk: 290 ± 8 g vs. 264 ± 12 g, P < 0.001; 18 wk: 552 ± 23 g vs. 473 ± 19 g, P < 0.001; 56 wk: 695 ± 58 g vs. 539 ± 25 g; P < 0.001).

**Oral glucose tolerance tests.** Fasting blood glucose and responses to intragastric glucose load were tested at 8, 20, and 52 wk in a separate set of naïve rats from the same shipment, which corresponds approximately with the acute microdialysis age groups. There was no statistical difference between the mean body weight of the actual experimental groups and the groups used in the OGTTs. At 8 wk, despite no strain difference in fasting blood glucose levels, OLETF had a slightly reduced glucose tolerance relative to LETO rats [at 30 min: 12.41 ± 0.73 mmol/l vs. 9.45 ± 0.90 mmol/l, (2) = 3.82, P < 0.05, n = 3] and an 12% increase in area under the curve (AUC). By 20 wk, prediabetes progressed dramatically in OLETF as indicated by a twofold rise in blood glucose levels at 30 min compared with LETO [14.02 ± 1.37 mmol/l vs. 7.99 ± 0.11 mmol/l, t(4) = 4.26, P < 0.01, n = 5]. Blood glucose levels remained significantly elevated through the 90-min sample [60 min: t(4) = 6.65, P < 0.01; 90 min: t(4) = 4.78, P < 0.01]. This response profile resulted in a 338% higher AUC in the OLETF group. Although one rat of the five tested was already diabetic at this point, the 20-wk cohort overall did not meet the criteria for overt diabetes. In contrast, at 52 wk, all OLETF rats developed diabetes with elevated fasting glucose levels [6.81 ± 0.83 mmol/l, t(4) = 2.29, P < 0.05, n = 5], a marked increase in their peak responses [19.9 ± 1.03 mmol/l, t(4) = 9.03, P < 0.001], and significantly elevated glucose levels at 120 min [14.39 ± 0.97 mmol/l, t(4) = 7.09, P < 0.01].

**Histology.** All microdialysis probes were placed within the caudomedial aspect of the NAC (A: 1.0–1.4 mm, L: 0.8–1.4 mm with respect to bregma and the midline, respectively). Because of the size of the probe membrane and the slightly varying ventral and medial position (possibly due to different brain size across age groups), we cannot be certain as to whether the samples originated exclusively from the medial shell or also from the adjacent core areas. Nonetheless, there were no statistically significant differences across samples regarding the probe position or placement between hemispheres.

**Extracellular DA concentration.** Two-way ANOVA revealed an overall effect of age (F_{2,45} = 92.680, P < 0.001) and strain (F_{1,45} = 9.899, P = 0.003) on basal extracellular concentrations of DA in NAC. The analysis of no-net-flux microdialysis data showed that DA levels (means ± SD) were 15.85 ± 5.63 nM and 20.10 ± 5.61 nM in 8-wk-old rats; 4.75 ± 1.25 nM and 7.37 ± 3.71 nM in 18-wk-old rats, and 2.39 ± 0.421 and 1.777 ± 0.405 nM in 56-wk-old LETO and OLETF rats, respectively (Fig. 1). Post hoc analysis demonstrated a significant difference between LETO and OLETF rats at 8 wk (P < 0.001), whereas at 18 wk, the difference barely missed the level of significance (P = 0.052). No statistical difference was noted at 56 wk (P = 0.748; Fig. 1).

**Extraction fraction of DA.** The extracellular fraction was affected by both age (F_{3,46} = 11.852, P < 0.001) and strain (F_{1,46} = 7.928, P = 0.007), with no significant interaction between age and strain (F_{3,46} = 0.855, P = 0.432). The actual extracellular fraction values were 0.514 ± 0.115 and 0.557 ± 0.066 at 8 wk, 0.538 ± 0.048 and 0.608 ± 0.096 at 18 wk, 0.527 ± 0.057 and 0.648 ± 0.049 at 56 wk, in LETO and OLETF, respectively (Fig. 2). Post hoc analysis between strains revealed a significantly increased extraction fraction in the 56-wk-old OLETF compared with LETO (P < 0.03) and also relative to 8-wk-old OLETF (P < 0.05). There was no difference between strains at 8 wk (P = 0.521) and 18 wk (P = 0.058), nor was there any difference in extraction fraction across all ages in LETO (Fig. 2).

**Potassium-induced DA release.** The reverse dialysis of aCSF containing 100 mM K⁺ (i.e., infused through the implanted probes) over a period of 20 min resulted in an increase
in extracellular DA outflow. Data from these tests are shown in Fig. 3.

Two-way ANOVA revealed an overall significant time (i.e., sample) \times strain interaction on the extracellular absolute DA levels in 18 wk \( (F_{8,169} = 2.189, \ P = 0.031) \) and 56-wk-old \( (F_{8,107} = 12.037, \ P < 0.001) \), but not in 8-wk-old rats \( (F_{8,153} = 1.928, \ P = 0.059) \). The magnitude of release, as well as the time dynamics of the effect, varied with age (Fig. 3). Post hoc analysis on the main effects showed that NAC DA release in response to potassium in 18-wk-old OLETF was higher than in LETO at 20 min \( (P = 0.001) \), but it lasted longer, with the strain difference being significant at 30 min after the onset of stimulation \( (P < 0.02; \text{Fig. 3B}) \). In 56-wk-old rats, the effect of stimulation on DA release reached higher levels in OLETF than LETO at 10 and 20 min \( (P < 0.02, \ P < 0.001, \text{respectively}; \text{Fig. 3C}) \). Subsequent one-way ANOVAs, however, demonstrated that in OLETF rats, stimulated DA release was higher than in LETO across all ages, including 8-wk-old rats. Specifically, at 8 wk, there was a significant strain difference of DA concentrations in the 5th fraction \( (F_{1,17} = 11.794, \ P = 0.003) \); at 18 wk, in the 5th and 6th fractions \( (F_{1,15} = 5.517, \ P = 0.033, \text{and } F_{1,18} = 5.043, \ P = 0.038, \text{respectively}) \); at 56 wk, in the 4th and the 5th fractions \( (F_{1,11} = 4.875, \ P = 0.049, \text{and } F_{1,12} = 22.495, \ P < 0.001, \text{respectively}) \).

When percent changes from baseline were analyzed, there were significant time \times age interaction in LETO \( (F_{16,209} = 18.433, \ P < 0.001) \) and OLETF rats \( (F_{16,207} = 55.434, \ P < 0.001) \).
DISCUSSION

In the present study, we sought to investigate basal and stimulated DA release in the NAC as a function of obesity and metabolic complications leading to type 2 diabetes in a rat model of hyperphagia-induced obesity, the OLETF rat. Our findings showed that basal and potassium-stimulated DA release, as well as DA clearance in the NAC of the obese OLETF rats, were different from the lean controls. However, the direction and the magnitude of these effects are age dependent corresponding with the development of obesity and insulin resistance.

Altered regulation of basal and stimulated DA levels in OLETF. In 8-wk-old animals, the extracellular DA concentrations were significantly higher in OLETF than in LETO rats. Then, both strains showed a continuous reduction of NAC DA throughout all time points tested with a profound drop in basal DA levels by 56 wk. At 18 wk of age, however, OLETF rats still demonstrated a trend (P = 0.052) of higher unstimulated extracellular DA compared with LETO. In OLETF, but not in LETO rats, this declining trend in extracellular DA concentration coincided with an increase in extraction fraction of DA, an index of uptake. Nonetheless, this measure was significantly higher in 56-wk-old OLETF rats compared with age-matched lean cohorts.

Findings from the potassium-stimulated release tests showed an increased sensitivity as a function of age in both strains. This observation reinforces previous reports using different methods (27, 65). Further analyses, however, demonstrated that in OLETF rats, both the amplitude and the duration of potassium-evoked DA release were augmented compared with LETO rats at all ages. This difference was apparent, even when the stimulated release was normalized with respect to differential ambient DA levels across strains and ages and indicates an increased vesicular capacity in obese OLETF.

An increase in depolarization-evoked DA release may be mediated by increased permeability of K⁺ channels and/or voltage-sensitive Ca²⁺ channels (18, 71). There is direct evidence that CCK redistributes intracellular Ca²⁺ or changes calcium influx through neuronal membranes (63, 78). It has also been demonstrated that modulation of methamphetamine-

![Fig. 4. Effect of infusion of high-potassium artificial cerebrospinal fluid (aCSF; 100 mM) on relative changes in microdialysate DA in the NAC of LETO (A) and OLETF (B) rats at different ages. For significant difference between age groups, P < 0.05: 56 vs. 8 wk; #56 vs. 18 wk; 118 vs. 8 wk.](http://ajpregu.physiology.org/DownloadedFrom/http://ajpregu.physiology.org)
induced DA release by CCK is highly \( \text{Ca}^{2+} \)-dependent (74). Thus, decreased expression of CCK-1 receptor may cause changes in \( \text{Ca}^{2+} \)-dependent components of neurotransmitter release. Independent of CCK, prediabetes in OLETF may also affect calcium-related DA homeostasis. Specifically, it has been shown that activity of calmodulin protein kinase II is altered under experimental hyperglycemic condition (59), with a potential effect on altered catecholamine release (58). In addition to hyperglycemia, diabetic keto-acidosis may promote monoamines sequestrations, which (22), in turn, cooperate on replenishing the vesicular pool of DA.

To our knowledge, only one study investigated DA release in the OLETF rat. Feifel et al. (23) reported that in OLETF rats, the amphetamine- and cocaine-induced DA release is altered. These effects, however, were region specific and showed an opposite direction, that is, an increased stimulation in the core and a reduced response in the shell region of NAC. As noted, the size of microdialysis probes used and the nature of placement for this type of study do not allow for differentiation between the core and shell; thus, a direct comparison between our results and Feifel et al.’s is impractical. Furthermore, Feifel et al. used the conventional microdialysis method, which does not account for potential differences in the absolute basal levels. Thus, studies using multiple distinct probe locations would be useful since a heterogeneity in CCK receptor distribution along the mediolateral and rostrocaudal axis of the NAC and multiplicity in affinity sites have been suggested with plausible role in CCK/DA interaction (3, 17, 36, 41, 70).

Increased vesicular capacity could be attributed to an altered function of the vesicular monoamine transporter, which is primarily responsible for vesicular packing. In fact, our observation that the extracellular levels of the DA metabolite DOPAC showed a consistent reduction following high-potassium stimulation suggests a shift from metabolism to storage. The finding that change in DOPAC levels did not differ between strains at any age, however, somewhat mitigates this proposition. Caution must be exercised though, since other critical variables such as precursor availability, synthesis, and DAT functions, may change differentially between strains over time, which, in turn, influence vesicular storage size (56).

Of particular interest is the accumulating evidence showing that insulin can regulate DAT. Specifically, insulin modifies phosphoinositol 3-kinase-mediated signaling leading to increased DAT expression on cell surface and \( [3\text{H}] \)-DA uptake (58). Thus, decreased expression of CCK-1 receptor may cause changes in \( \text{Ca}^{2+} \)-dependent components of neurotransmitter release. Independent of CCK, prediabetes in OLETF may also affect calcium-related DA homeostasis. Specifically, it has been shown that activity of calmodulin protein kinase II is altered under experimental hyperglycemic condition (59), with a potential effect on altered catecholamine release (58). In addition to hyperglycemia, diabetic keto-acidosis may promote monoamines sequestrations, which (22), in turn, cooperate on replenishing the vesicular pool of DA.

Effect of aging on DA and development of diabetes in OLETF rats. In this study, aging played a critical role in the manifestation of strain differences observed in DA regulation between the OLETF and LETO rats. In general, it has been known for some time that aging results in a reduced number of DA neurons, as well as impaired DA functions in various brain areas of both human and rats (47, 60). It has also been demonstrated that extensive reduction in the number of DA neurons in the SN is also causing diminished DA levels in the striatum and the basal ganglia, and, in turn, motor deficits characteristic to Parkinson’s disease (37). Despite this information, to our knowledge, this is the first study demonstrating aging’s diminishing effect on the actual DA concentration in the NAC of rats in vivo using quantitative microdialysis. Nonetheless, the current study cannot differentiate between strain effect of age in the absence of increased adiposity and metabolic abnormalities.

Potential relationship between CCK-1 receptors and altered DA regulation. There is evidence showing a direct influence of CCK-ergic transmission on DA neuronal functions (3, 17). CCK is colocalized in a subpopulation of DA neurons of the VTA and SN projecting to the limbic and basal ganglia structures, including the NAC (36). It regulates the firing rate of the DA neurons and also the DA release in terminal regions (3). CCK and CCK-ergic drugs variously affect DA neurotransmission in the brain due to heterogeneity in CCK receptor population and difference in CCK receptor distribution (61). Disruption of CCK-1 mediated neurotransmission by the CCK-1 receptor antagonist devazepide has been reported to elicit an increase in DA content of the NAC (44). Of particular interest, unlike the CCK-2 receptors, which are abundant in the forebrain, CCK-1 receptors are expressed only in select brain structures, particularly those involved in reward and motivation (34). In this context, it is plausible that the lack of functional CCK-1 receptors in the OLETF rats may also result in behavioral alterations that are related to CCK/DA interaction (3, 61).

For example, Bednar et al. (2) demonstrated the potency of DA D1 or D2 receptor antagonists to augment the inhibitory effect of CCK on intraorally infused sucrose. Recently, we showed that OLETF rats were more sensitive to peripheral administration of D1 or D2 receptor antagonists in reducing sucrose intake (20). Thus, it seems that in the OLETF rats, an altered DA regulation may indeed contribute to the changes in feeding behaviors observed in this strain (i.e., increased sweet preference and overeating, in general).

In this study, we have expanded the investigation of the relationship between CCK-1 receptors and DA in the NAC to include corollaries of dietary obesity and late-onset diabetes. Specifically, on the basis of our observation that in OLETF rats, both changes in the basal and phasic DA release are accentuated by age, one may assume that CCK-1 receptors may play a permissive role in maintaining DA release. This, together with an increased cytosolic pool (i.e., inferred from increased reuptake) supplying DA to the releasable pool, may explain sustained or even increasing capacity of the terminals to release more DA in response to depolarization in the diabetic OLETF rats. Thus, it is not impossible that an augmented phasic DA signaling in response to stimulation with palatable meals may contribute to overeating in this strain.
homeostasis against challenges by environmental factors. In this context, one may explain the observed alterations in the DA system of the OLETF rats as a result of developmental adaptation to their deficient CCK-1 receptors. An alternative explanation could be that DA regulatory deficits in OLETF rats are rather secondary to their hyperphagic phenotype downstream from the mutant genotype. In the first case, however, DA alterations would be expected to facilitate eating, whereas in the latter case, would result in curbing appetite. Although the exact role of DA in behavioral reward, in general, and its relationship with eating, in particular, even in nonobese subjects, is far from being settled (4), accumulating evidence has linked increased NAC DA levels with increased eating. For example, stimulation that results in DA release in the NAC also stimulates food consumption (29, 33) and intake of sucrose solution (30). Further evidence for a positive correlation between tonic level of DA and food consumption had been shown in DAT knockout mice. Particularly, these mice express high basal DA levels, and similar to the OLETF rats, not only do they have an increased meal-size and sucrose consumption, but they are also heavier than wild-type mice (55). Although the DAT knockout mice demonstrate a retarded growth that makes food intake and body weight less comparable with wild-type mice, these mice also express an increased avidity for sucrose (15). These and other pieces of evidence, together with our present findings, suggest that the altered DA regulation, particularly the increased basal DA levels, seen in the OLETF rats occurring very early in their lives, even before marked increase in body weight or deficits in glucose control develop, are rather contributory to increased eating than a consequence of it. Indeed, it has been shown recently that OLETF rats are hyperphagic as early as the first postnatal day (75) pointing to a critical prenatal challenge DA homeostasis. Whether these effects are direct or indirect with respect to the DA systems, as well as to the behavior, remains to be elucidated.

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