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Altered dopamine D2 receptor function and binding in obese OLETF rat

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Abstract

A decrease in D2-like receptor (D2R) binding in the striatum has been reported in obese individuals and drug addicts. Although natural and drug rewards share neural substrates, it is not clear whether such effects contribute also to overeating on palatable meals as an antecedent of dietary obesity. Therefore, we investigated receptor density and the effect of the D2R agonist quinpirole (0.05, 0.5 mg/kg, S.C.) on locomotor activity and sucrose intake in a rat model of diet-induced obesity, the CCK-1 receptor-deficient Otsuka Long Evans Tokushima Fatty (OLETF) rat. Compared to age-matched lean controls (LETO), OLETF rats expressed significantly lower [¹²⁵I]-iodosulpride binding in the accumbens shell (−16%, $p < 0.02$). Whereas the high dose of quinpirole increased motor activity in both strains equally, the low dose reduced activity more in OLETF. Both doses significantly reduced sucrose intake in OLETF but not LETO. These findings demonstrate an altered D2R signaling in obese OLETF rats similar to drug-induced sensitization and suggest a link between this effect and avidity for sucrose in this model.

Keywords

overeating; palatability; reward; striatum; cholecystokinin; type-2 diabetes

1. Introduction

Dopamine (DA) D2-like receptors (D2Rs) have been implicated in the pathology of eating [23] and associated with obesity [5,42] and drug addiction [34,46]. In particular, brain imaging studies have documented reductions in D2 receptors in the striatum of obese individuals [45] and D2 receptor density was inversely related to the body mass index (BMI). In contrast, PET studies in patients with anorexia nervosa have reported higher than normal striatal D2 receptors availability [23]. These and other observations suggest an intricate relationship between DA signalling and the pathology of eating and body weight maintenance [44]. Animal models of obesity have provided further evidence of this relationship. For example, combined treatment with DA D1 and D2 receptor agonists (e.g. SKF3893 and bromocryptine) in leptin deficient ob/ob mice reduces hyperglycemia, hyperphagia, hyperinsulinemia and elevated body weight

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associated with this strain, suggesting that decreased DA signaling is causally related to the phenotype [11,43].

Otsuka Long-Evans Tokushima Fatty (OLETF) is a naturally occurring cholecystokinin (CCK)-1 receptor knockout rat strain due to a 6.8-kb deletion in the CCK-1 receptor (CCK-1R) gene. This rat is hyperphagic, gradually develops obesity and diabetes, which resembles human non-insulin-dependent diabetes mellitus (NIDDM) [28]. CCK-1Rs are the receptor subtype that mediate CCK's actions in satiety [31]. 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 lean background strain, the Long Evans Tokushima Otsuka (LETO) rats. Furthermore, OLETF rats have deficits in responding to CCK, and gastric and intestinal preloads [16,32]. The underlying cause(s) of hyperphagia in this rat model has not been fully understood. The overall hyperphagia, however, suggests that OLETF rats may have additional deficits beyond that of controlling meal size. For example, obesity as well as NIDDM can be greatly reduced by caloric restriction [36] or exercise [41] suggesting that obesity and NIDDM in OLETF rats are secondary to their hyperphagia. Relevant to this, we have demonstrated that in addition to diminished sensitivity to postingestive satiation signals, OLETF rats express increased sham intake of normally preferred sucrose solutions [18] and an increased avidity generalized to various agents that taste sweet to human including non caloric sweetener saccharin and the amino acid alanine [25]. This finding suggests that an increased sensitivity to sweet reward may be contributory to the development of obesity in this strain.

Co-release of CCK and DA as well as co-localization of CCK and DA receptors in the striatum has been demonstrated [27]. Whereas CCK-2 receptors are abundant in various regions of the striatum and exert an inhibitory control over DA release, CCK-1Rs occur predominantly in the shell region of the nucleus accumbens (NAcc) and stimulate DA release [29,38]. Of particular importance, it has been suggested that CCK interacts with DA to control sucrose intake [7] and in motor sensitization effect of psychostimulants [8]. Thus it is reasonable to assume that in OLETF rats, the lack of control from CCK-1Rs may result in adaptive changes in DA transmission with behavioral consequences leading to the development of obesity.

In support of this notion, there is evidence showing that the DA system in OLETF is more sensitive to stimulation by psychostimulants [22] and also by long-term sucrose feeding [24]. Furthermore, our laboratory demonstrated that in OLETF rats access to sucrose differentially modulates prepulse inhibition and acoustic startle response [17]. These findings suggest that OLETF rats have an altered DA regulation that may contribute to the obese behavioral phenotype (i.e. overeating, and increased sensitivity to palatability), however DA receptor functions in this strain have not been directly tested. Therefore, the present study was aimed at investigating behavioral sensitivity of D2Rs in prediabetic, obese OLETF rats using systemic injections of the selective D2R agonist quinpirole, and assessing the binding density of the D2Rs in various brain areas related to reward and psychostimulant sensitization.

2. Materials and methods

2.1. Subjects

At the beginning of the experiments, male OLETF and LETO (Otsuka Pharmaceuticals, Japan) rats were 24 weeks old, with an initial weight of 560–585g and 430–450g, respectively. All rats were maintained on ad libitum standard laboratory chow (Global Diet-2018, Harlan Teklad) and tap water, unless otherwise noted. Animal protocols were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University, College of Medicine, and were in accordance with NIH guidelines.

2.2. Substances used

(-)-Quinpirole hydrochloride (Tocris Cookson, Inc.) was dissolved in physiological saline and injected subcutaneously (SC) at doses of 0.05 mg/kg (“low dose”) and 0.5 mg/kg (“high dose”) before placing the animal in the open-field arena or 20 min prior to the presentation of sucrose in intake tests. Sucrose (Fisher Scientific) was diluted in filtered tap water, and used at a concentration of 0.3M (~10% w/v) that is palatable to rats.

2.3. Experiments and procedures

2.3.1. Experiment 1: Activity tests—Open field tests were conducted in 10 OLETF and 10 LETO rats using an automated activity monitoring system (TruScan, Coulbourn Instruments). Following two 60-min habituation training sessions, 120 min daily sessions were recorded for 5 consecutive days. On Days 1, 3, and 5 animals received SC saline injection immediately before their placement in the open field arena. Day 1 served as baseline, whereas Days 3, and 5 served as “wash-out” periods for low and high doses of quinpirole injections, respectively. On Day 2, animals were injected with the lower, 0.05mg/kg dose of quinpirole followed by the higher, 0.5mg/kg dose of quinpirole on Day 4. All animals were maintained on ad libitum access to chow and water in their home cages, whereas no food and water was available in the open field arena during testing. Activity data for the first 20 minutes were excluded and the 20 to 120 minutes time interval was analyzed. Parameters measured were the following: ambulatory activity (horizontal beam crossing), time spent in the center of the arena; vertical exploratory behavior (number of rears), and stereotypy episodes (defined as consecutive non-forward, non-vertical movements occurred within less than 2 seconds delay and within a defined square field).

2.3.2. Experiment 2: One-bottle sucrose intake—After locomotor activity tests in Experiment 1, animals were maintained in their individual home cages for about 2 weeks with no treatment or testing except daily readings of intakes and body weight. Following a 2 day habituation to daily 30 min access to 0.3M sucrose (1000h-1030h) presented in graduated plastic cylinders (1 ml reading accuracy) a 5-day quinpirole/vehicle regimen similar to Experiment 1 was performed except that rats received their respective injections 20 min prior to sucrose presentation. Food and water was present throughout the experiment and 24 h food and water intake and body weight were measured daily including the test days and analyzed for drug effects.

2.3.3. Experiment 3: Receptor binding assays—Naïve OLETF and LETO rats (n=5 each strain) of identical age (24–28 wks) were sacrificed by decapitation and brains were removed and immediately immersed in –40°C isopentane (2-methylbutane) and stored at –70°C. Twenty µm coronal brain sections were cut using a cryostat and thaw-mounted on polylysine coated slides. The brain regions examined were from the dorsal and ventral striatum (1.7 to 1.1mm from Bregma; inclusive of the nucleus accumbens (NAcc) and dorsal striatum [19]). Four sections from each brain region were serially mounted on a slide. Approximately 8 slides were taken from each brain region (representing an approximate distance of 160µm between sections). One slide from each brain region was subject to cresyl Lecht violet staining to accurately determine anterior-posterior anatomical coordinates for proper analytical comparisons. The remaining sectioned tissue was kept at –70°C until assayed. For autoradiography, we used [125 I]-iodosulpride (with Kd of 0.6nM and 1.2 nM for D2 and D3, respectively; NEN/PerkinElmer, specific activity 2000 Ci/mmol) D2R subtype ligand binding. Tissue preparation and quantitative analysis were identical to those used previously in our lab [9].

2.3.4. Oral glucose tolerance test (OGTT)—To screen for diabetic status, an oral glucose tolerance test was performed in a subset of rats (n=5, per strain) used in Experiments 1 and 2

one week prior to the open field tests, and in all subject prior the histological study (Experiment 3). The test was administered following a 16hr fast, when an oral glucose load (2g/kg) was delivered to each rat orally via latex gavage. Blood glucose was measured before and at 30, 60, 90, and 120 min post-glucose loading by a standard glucometer (LifeScan, One-Touch Basic). Animals were classified as diabetic if the peak level of plasma glucose was ≥ 300 mg/dL and a peak glucose level at 120 min > 200 mg/dl [28].

2.4. Statistical analyses

One-way ANOVAs were performed for comparisons of baseline parameters between strains. The effect of drug injection, strain and drug/strain interaction on behavioral parameters in all experiments was analyzed with two-way ANOVAs. A post-hoc Dunnett's test was performed when ANOVA showed significant difference. Binding density and blood glucose levels in OLETF and LETO rats following an OGTT were compared using planned t-tests. All data were expressed as means + SEM. Differences were considered statistically significant if $P < 0.05$. Statistical analyses were computed with Statistica 6.0 software (Tulsa, OK).

3. Results

3.1. Experiment 1: Open-field tests

Figure 1 summarizes the main findings from the open field tests. Under baseline condition (i.e. vehicle injection, Fig1A), OLETF and LETO rats' ambulatory activity (i.e. horizontal beam crossing) was statistically identical, despite that OLETF rats showed a trend for decreased movement (-12% , N.S.). Two-way ANOVAs, however, showed a significant drug [$F(1,86) = 8.67$, $p < 0.01$], and strain effect [$F(1,86) = 51.48$, $p < 0.001$] and treatment $\times$ strain interaction [$F(4,46) = 23.9$, $p < 0.001$]. Post hoc tests revealed that while the low dose of quinpirole decreased animals' horizontal activity in both strains compared to baseline (OLETF: $p < 0.01$; LETO: $p < 0.05$), this effect was significantly more pronounced in OLETF than LETO rats (OLETF vs. LETO: $p < 0.05$). The high dose induced locomotor hyperactivity in both strains ($p < 0.01$), (Fig. 1A). There was no significant post hoc effect between saline treatments when Day 3 and Day 5 were compared.

Further analysis showed that in baseline condition OLETF rats spent less time in the center area than LETO did [4350 ± 213.8 s vs 2810 ± 282.7 s; $F(1,12) = 83.22$, $p < 0.001$]. Although quinpirole injection had an overall influence on this measurement [drug $\times$ strain interaction: $F(4,86) = 2.49$, $p < 0.05$], the effect was dose and strain dependent. Specifically, quinpirole increased time spent in the center only in LETO rats when injected with the higher dose [5392 ± 146.9 s, $p < 0.01$], whereas in OLETF quinpirole had no effect on this measure at either dose [low dose: 2742 ± 365.9 s, NS; high dose: 3125 ± 350.5 s, NS].

Under basal condition, there was no statistical difference in vertical movements, i.e. rearing, between OLETF and LETO rats (Fig. 1B). However, quinpirole significantly altered the number of entries into vertical position [$F(4,86) = 6.62$, $p < 0.001$] and also there was a significant drug $\times$ strain interaction [$F(4,86) = 3.47$, $p < 0.05$]. While low dose quinpirole significantly and equally reduced rearing in both strains ($p < 0.05$), the higher dose increased rearing exclusively in LETO rats ($p < 0.05$).

When stereotypies were analyzed, two-way ANOVAs demonstrated a significant effect for treatment [$F(4,86) = 15.82$, $p < 0.001$] but no interaction or strain effect. Post hoc tests revealed that the low quinpirole dose significantly reduced the number of stereotypy episodes during 100 min test in both OLETF and LETO rats ($p < 0.01$). In contrast, the high dose had no effect in either strain.

3.2. Experiment 2: Ingestive responses

There was a significant difference in 30-min one-bottle sucrose intake between OLETF and LETO rats [$F(1,68)=32.36$, $p<0.001$]. Baseline sucrose intake was almost twice in OLETF compared to LETO [$+99.0 \pm 13.95\%$, 17.3 ± 1.2 ml vs. 8.7 ± 0.9 ml; $p<0.01$; Figure 2]. Quinpirole had an overall effect on sucrose intake [$F(3,68)=20.07$, $p<0.001$] and there was a significant interaction between strain and drug [$F(3,68)=3.21$, $p<0.05$]. Quinpirole at low dose significantly reduced sucrose intake relative to vehicle baseline in OLETF [$-19.4 \pm 8.8\%$, $p<0.05$], but had no effect in LETO rats. In contrast, the high dose of quinpirole significantly reduced sucrose intake in both strains (OLETF: $-67.5 \pm 6.2\%$; $p<0.01$; LETO: $-44.2 \pm 16.6\%$, $p<0.05$, compared to saline baselines).

After sucrose intake tests, animals received free access to regular chow and water, and 24-hr intakes were measured. Under baseline conditions, although OLETF rats tended to consume more daily chow than LETO (29.86 ± 5.46 g vs. 24.62 ± 5.01 g), this difference was not statistically significant. However, two-way ANOVA showed a highly significant effect for drug [$F(4,80)=11.43$, $p<0.001$] as well as for strain $\times$ drug interaction [$F(4,80)=5.37$, $p<0.001$]. Post hoc tests revealed that whereas the low dose had no effect on chow intake in either strain, the higher dose significantly reduced food consumption only in OLETF rats (-44.1% , $p<0.01$).

Similar to food intake, there was no strain difference in 24 hr water intake following saline injection and sucrose tests (OLETF: 36.25 ± 11.13 ml, LETO: 35.11 ± 12.94 ml), but the total fluid intake, i.e. when water combined with 30-min sucrose intake was significantly higher in OLETF ($+18.3\%$, $p<0.05$). Furthermore, quinpirole significantly altered water intake [strain $\times$ drug interaction: $F(4,79)=7.23$, $p<0.001$]. The effects of the drug was carried by a significant reduction of water intake in OLETF rats after both doses of quinpirole injections [low dose: -44% ; high dose: -51% , $p<0.01$ for both comparisons]. In contrast, quinpirole had no effect on water intake in LETO at either dose.

3.3. Experiment 3: Receptor binding density

The results of the [125 I]-iodosulpride radioligand binding assays are summarized in Fig. 3 and depicted as percent of controls. We observed a significantly lower D2R binding in the NAcc shell of the OLETF compared to age-matched lean LETO rats ($-16.4 \pm 4.2\%$, 18.96 ± 0.96 nCi/mg vs. 22.69 ± 1.34 nCi/mg; $t=2.74$, $p<0.02$). In contrast, none of the other brain areas investigated showed significantly different binding. The absolute density values (nCi/mg tissue equivalent) for OLETF and LETO, respectively were the following: NAcc Core: 21.06 ± 1.0 , 21.22 ± 0.39 ; dorso-lateral striatum: 37.32 ± 0.88 , 37.51 ± 1.24 ; substantia nigra pars compacta: 8.38 ± 0.58 , 9.40 ± 1.03 ; VTA: 7.91 ± 0.26 , 8.19 ± 0.60 .

3.4. Oral glucose tolerance

OLETF rats used in both the behavioral and histology experiments showed increased blood glucose levels relative to LETO in response to acute oral glucose challenge. Significant increases were observed in OLETF rats at 30 min and 60 min ($P<0.01$ for both time points) compared to LETO rats, with highest blood glucose peak at 30 min. It is important that OLETF rats did not meet the criterion for overt diabetes in any test [28]. Thus OLETF rats across all 3 experiments were prediabetic.

4. Discussion

4.1. Differential strain effects of D2 agonist on activity

In the activity tests, there was a biphasic effect by the D2R agonist quinpirole in respect to the dosage. Whereas low-dose (0.05 mg/kg) quinpirole decreased, the high-dose (0.5 mg/kg) increased various measures of horizontal and vertical activity. These effects were, however,

different between strains. The inhibitory effect of low-dose quinpirole was significantly higher in OLETF than in LETO rats. Whereas the low dose (0.05mg/kg) of quinpirole decreased, the high dose (0.5mg/kg) increased ambulatory activity.

The doses used in the present study were chosen based on data demonstrating differential stimulus properties of a pre- vs. a putative postsynaptic dose of quinpirole at 0.05 mg/kg and above 0.2mg/kg, respectively [15,47]. Furthermore, the use of two doses of quinpirole in our experiments was neither intended nor sufficient to quantitate sensitivity shift in this strain. Rather, our aim was to reveal potential alterations in the D2 receptors of this obese strain at either the pre-, or the postsynaptic domain, or both. Our findings are in concert with earlier reports showing that behavioral responses to acute peripheral quinpirole injection are biphasic, time and dose dependent [20]. Low dose of quinpirole (< 0.1 mg/kg) induces behavioral inhibition, while higher doses elicit a biphasic response; brief decrease in behavioral activity followed by a period of hyperactivity. A low dose of quinpirole can act preferably on DA D2 presynaptic receptors, which inhibits DA release, while higher dose activates both pre- and postsynaptic receptors, eliciting increased response of postsynaptic DA receptors [15,20,47]. On the other hand, a more pronounced inhibition on OLETF rats' activity by low-dose of quinpirole is likely to be a result of increased response of presynaptic D2R receptors.

Although the exact nature of differential effect of quinpirole on time spent in the central arena between strains are not clear, the baseline difference suggesting increased anxiety in OLETF rats replicates previous findings [48]. The abolished capacity of high dose quinpirole to increase rearing in OLETF rats might indicate a reduced exploratory response. Nevertheless, disrupted correlations between horizontal and vertical activities in the open field have been suggested to reflect profound behavioral disruption due to altered DA control of the cortico-striatal mechanisms [33].

4.2. Differential strain effects of D2 agonist on sucrose intake

Quinpirole at the high dose reduced intake of 0.3 M sucrose solution and 24 hour food consumption in OLETF, but not LETO rats. Although the specific contribution of different classes of DA receptors to ingestive responses has been debated, decreased intake of rapid sucrose or increased aversion to a more concentrated sucrose solution after acute DA agonist treatment has been reported [14,40]. Furthermore, quinpirole has been demonstrated to produce a dose-dependent conditioned taste aversion (CTA) in rats [1]. Although CTA was not directly tested in the present experiment, an effect from aversive properties of quinpirole on sucrose intake cannot be fully excluded. However, the finding that sucrose intake did not differ on saline days between and after drug treatment days together with the observation that in LETO rats even the high dose of quinpirole did not reduce sucrose intake mitigate the possibility of such confound in our design. The present findings are also in concert with our previous observation that reverse microdialysis of D2R antagonist into the NAcc of normal, lean rats prior to 0.3M sucrose presentation results in both DA release and concurrent stimulation of sucrose intake [26]. These findings collectively support a role for D2Rs in regulation of sucrose intake based on its rewarding properties.

In the present experiment, both quinpirole doses reduced 24h water intake in OLETF, but not in LETO rats. Unlike in food intake, evidences for DA's role in water intake is scarce, nonetheless a decreased fluid consumption after acute quinpirole injection has been also reported [12]. It is noteworthy that an increased avidity to water in OLETF rats was observed earlier [17], and an increased overall fluid consumption was also apparent in the present experiment. Thus, one may suppose that similar to increased sucrose and food intake, D2R mechanisms may also be involved in overall excessive fluid intake in this strain.

4.3. Strain differences in D2R binding density

The D2R binding assays revealed a decreased density in the NAcc shell in naïve OLETF rats that were identical with those used in the behavioral experiments with regards to their age, body weight and glycemic status. It is intriguing, however, that D2R binding in other investigated areas including the core and dorsolateral striatum which have been primarily associated with incentive learning and motor sensitization was unaffected. Among several possibilities, this may suggest dissociation between increased functional sensitivity (i.e. inferred from the pronounced motor effects of quinpirole in OLETF rats) and expression of the D2Rs potentially as a function of the input/output characteristics of the area investigated, as well as with respect to different aspects of the behavior. Although it is tempting to interpret the NAcc shell's involvement in the context of differential expression of CCK-1Rs and CCK-2Rs across subdivisions of the NAcc, in a previous study using an identical method, we observed a similar reduction in D2R binding in the NAcc of normal (i.e. non-mutant, Sprague-Dawley) lean rats following as short as 6 days intermittent brief daily exposures to 0.3M sucrose [10]. Interestingly, in that experiment, lean rats with reduced D2 availability not only did binge on palatable sucrose, but their daily intake of regular chow increased as well. This observation may explain why OLETF rats also overeat on their maintenance diet and that quinpirole treatment affected food and water intake in this strain but not in lean controls.

4.4. Potential mechanisms

What mechanisms can lead to the development of altered D2R signalling? Once a natural stimulus becomes familiar after repeated presentation, the resultant DA response becomes blunted [6]. In contrast, palatable food stimuli including intermittent sham feeding on sucrose repeatedly results in DA release in the NAcc [4] imposing a sustained regulatory challenge to the synaptic apparatus. In an environment where highly palatable diets are abundant and freely available, such stimulation (i.e. repeated and higher DA release) may result in sensitization to the diets' behavioral stimulant properties. A similar priming mechanism has been proposed for the effects of drugs of abuse [19] and various pathological states associated with altered DA release [39]. In this context, one may assume that repeated exposure to highly palatable meals also may, in turn, contribute to development of lasting eating habits including cravings in humans [37] or sugar bingeing in rats [10,13]. Although a direct analogy between drug and food addictions have been debated, intermittent access to sugars in rats, in fact, has been shown to elicit behavioral signs of dependence [10,13], and locomotor cross-sensitization with amphetamine [3].

Based on this, it may be hypothesized that once neuroadaptation to the D2 signalling develops, irrespective of the triggering event (e.g. chronic stimulation with highly palatable diet, or drug of abuse, etc.) the reward system becomes sensitized also to other rewarding stimuli. In support of this is the example that sugar bingeing rats also express augmented intake of alcohol [2]. Similarly, CCK-1R deficient mice increase alcohol intake concurrent with a decrease in D2R immunoreactivity in the NAcc shell [30].

The present findings together with further DA-related deficits described earlier in the OLETF rats (e.g., learning, memory, motor, and sensory-motor gating deficits [9,17,21,35]) and with the suggested role of CCK in behavioral sensitization to psychostimulants [8] supports the notion that altered sweet preference and overall augmented ingestive responses may represent a component of a more generalized increase in sensitivity of the reward system in this obese rat model.

4.5. Conclusions

On the basis of the present findings we conclude that prediabetic, obese OLETF rats express increased behavioral sensitivity to exogenous stimulation with D2R agonist, and a reduced

D2R density predominantly in the NAcc shell that has relevance to reward guided behaviors, including food reward. Thus, it is feasible that a similar increased sensitivity to endogenous ligand upon stimulation by palatable foods may contribute to overeating in this strain. Since a similar deficit in DA functions have been shown in other obese animal models and human as well as drug addicts, it may represent a common mechanism of increased susceptibility for reward seeking behaviors including predisposition to diet-induced obesity in some individuals.

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Fig. 1.

Effect of quinpirole on ambulatory (A) and vertical (B) activity of prediabetic, obese OLETF and age-matched lean LETO rats. Data depict means $\pm$ SE of beam crossing during a 100-min session, 20 min following injection of vehicle (0 mg/kg, S.C.) or quinpirole (0.05 or 0.5 mg/kg, S.C.). * $p < 0.05$, LETO to vehicle vs. 0.05 mg/kg drug; ** $p < 0.01$ LETO to vehicle vs. 0.5 mg/kg drug; # $p < 0.01$, OLETF to vehicle vs. 0.05 mg/kg drug; ## $p < 0.01$, OLETF to vehicle vs. 0.5 mg/kg drug; † $p < 0.05$, OLETF vs. LETO to 0.05 mg/kg.

Fig. 2.

Effect of quinpirole on sucrose intake in prediabetic, obese OLETF and age-matched lean LETO rats. Data depict means $\pm$ SE of thirty-minute intake of palatable, 0.3M sucrose solution, presented 20 min following injection of vehicle (0 mg/kg, S.C.) or quinpirole (0.05 or 0.5 mg/kg, S.C.). * $p < 0.05$, LETO to vehicle vs. 0.5 mg/kg drug; # $p < 0.05$ OLETF to vehicle vs. 0.05 mg/kg; ## $p < 0.01$, OLETF to vehicle vs. 0.5 mg/kg drug; †† $p < 0.01$, OLETF vs. LETO to vehicle.

Fig. 3.

[125I]-iodosulpride radioligand binding in various brain regions of prediabetic obese OLETF rats. Brain regions examined were the caudal-medial shell of the nucleus accumbens (Shell), the core of the nucleus accumbens (Core), dorsolateral striatum (DL-STR), the pars compacta of the substantia nigra (SN pc), and the ventral tegmental area (VTA). Data reflect means $\pm$ SE and are expressed as percentage of age-matched, lean LETO controls (dotted line = 100%). # ($p < 0.02$).