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BRIEF COMMUNICATION

Cortical [^{3}H]Ketanserin Binding and 5-HT$_{2A}$ Receptor-Mediated Behavioral Responses in Obese Zucker Rats

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CHAOULOFF, F., I. COUPRY AND V. BAUDRIE. *Cortical [^{3}H]ketanserin binding and 5-HT$_{2A}$ receptor-mediated behavioral responses in obese Zucker rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 309-312, 1995.—Past studies have indicated that genetically obese Zucker (fa/fa) rats are hypercorticoid, and that this neuroendocrine alteration plays a key role in the syndrome. In keeping with the proposal that glucocorticoids may upregulate central 5-HT$_{2A}$ receptors, we have studied the effects of acute and repeated 5-HT$_{2A}$ receptor stimulation by 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane (DOI) in lean and obese Zucker rats. Acute injection of DOI (2 mg/kg, SC) elicited a lower number of head shakes in obese rats compared to that measured in lean rats. Conversely, neither DOI-elicited decreases in food intakes and body weights nor cortical [^{3}H]ketanserin binding were affected by obesity. In rats repeatedly pretreated with DOI, biochemical and functional indices of 5-HT$_{2A}$ receptor downregulation failed to reveal an effect of obesity. It is suggested that 5-HT$_{2A}$ receptor-mediated functions, but not their downregulation, may be differentially affected in the hypercorticoid obese Zucker rat.

Obese Zucker rats 5-HT$_{2A}$ receptors DOI Head shakes Food intake [^{3}H]Ketanserin binding

THERE is a great deal of evidence for a control of central serotonergic systems by glucocorticoids [see (5) for a review], and the well-documented negative influence of glucocorticoids upon 5-HT$_{1A}$ receptors provides one good illustration of this knowledge. However, studies related to the influence of glucocorticoids upon another subtype of 5-HT receptors, namely the 5-HT$_{2A}$ subtype [previously called 5-HT$_2$, see (11)], have yielded divergent data. For instance, glucocorticoid removal by means of adrenalectomy has been reported to increase 5-HT$_{2A}$ receptor binding in the hippocampus (19), but not in the hypothalamus (19) or cortex (6,15). This is also true for 5-HT$_{2A}$ receptor function, as some authors (15), but not others (6), have reported a decreased body/head shake response to 5-HT$_{2A}$ receptor stimulation in adrenalectomized rats. As far as the intrinsic effects of glucocorticoids upon 5-HT$_{2A}$ receptors are concerned, data are contradictory; thus, cortical [^{3}H]ketanserin binding (and 5-HT$_{2A}$ receptor-mediated wet dog shakes) have been reported to be increased by repeated administration of glucocorticoids (15), but this observation was not shared by others (12). Actually, the aforementioned discrepancies probably arise from the different paradigms that were used, including differences related to the dose of glucocorticoid used and/or the duration of treatment.

One means to circumvent the pitfalls outlined above could rely upon a measure of the biochemical and functional properties of 5-HT$_{2A}$ receptors in (patho)physiological models of hypercorticism. Indeed, numerous data strongly suggest that the genetically obese Zucker (fa/fa) rat could be such a model. Firstly, different studies have shown that obese (fa/fa) rats display chronic hyperactivity of the hypothalamo-pituitary-adrenal (HPA) axis, compared to their lean (Fa/Fa or Fa/fa) littermates (7,9). Secondly, some of the phenotypic character-

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istics of the obese Zucker rats (i.e., hyperinsulinemia, hyperphagia, obesity, and triglyceridemia) may be reversed or attenuated by glucocorticoid removal and reinstated by corticosterone replacement [for a review see (4)].

In keeping with these data, we have used the aforementioned behavioral (i.e., head shake response to the acute stimulation of 5-HT_{2A} receptors) and biochemical (i.e., binding properties of cortical 5-HT_{2A} receptors) tools to get an insight into the impact of obesity-related hypercorticism upon 5-HT_{2A} receptors. In addition, the hypophagic response to the acute stimulation of 5-HT_{2A} receptors (21) was also measured to test whether putative alterations in the head shake behavior applied to other 5-HT_{2A} receptor-mediated behaviors. Lastly, because rat brain 5-HT_{2A} receptors rapidly downregulate upon repeated stimulation (16), we have used all these tools to examine whether obesity-related hypercorticism had an impact on 5-HT_{2A} receptor downregulation.

METHOD

Animals

Lean (Fa/Fa or Fa/fa) and obese (fa/fa) male Zucker rats, aged 10 weeks, were obtained from the Zucker colony at IFFA CREDO (Les Oncins, France). The rats were housed in pairs (lean with lean and obese with obese, respectively) with food and water ad lib, in an animal quarter maintained at 21 ± 1°C with a 12L : 12D cycle (lights on at 0800 h). The rats were kept under these conditions for 1 week before testing (final weights 309 ± 4 g and 396 ± 6 g for lean and obese rats, respectively).

Procedures

Rats were injected three times with either 0.9% saline (group 1) or 2 mg/kg (SC in 1 ml/kg) of the 5-HT_{2A}/5-HT_{2C} receptor agonist 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane [DOI; see (10)] (R.B.I., BioBlock, Illkirch, France) (group 2), according to a slight modification of the procedure initially described by Leysen et al. (16). Thus, our protocol involved three injections within 24 h: the first was administered between 0900 and 1100 h (first day), the second between 1800 and 2000 h (first day), and the third between 0900 and 11:00 h (second day). Rats (n = 40) and amounts of food remaining in the dispensers (n = 20) were weighed 30 min before each injection, but also between 1800 and 2000 h on the second day (to get an insight on the effects of the third injection).

Five minutes before the first and third injections (i.e., during two consecutive mornings), rats from group 2 were transferred from their home cages to individual plastic observation cages (24 × 24 × 30 cm). Then the animals were injected with DOI (2 mg/kg, SC), replaced in their cages, and left undisturbed for 3 min. The number of head shakes was then counted for 30 min. Rats were observed in pairs with one lean rat and one obese rat. Rats from group 1 were repeatedly injected with 0.9% saline according to the protocol defined above, and replaced in their home cages. On the first and third treatments with 0.9% saline, food and water were removed from the home cages for 35 min. Twenty-four hours after the third injection (i.e., in the morning of the third day), nine lean and nine obese rats that had received either three injections of 0.9% saline or three injections of DOI were killed, and the frontal cortices were rapidly dissected on an ice plate and stored at −80°C. 5-HT_{2A} receptor binding was performed using [^{3}H]ketanserin (specific activity: 63.7 Ci/mmol; Dupont/NEN, Les Ulis, France) and methysergide maleate (10 μM; R.B.I., BioBlock, Illkirch, France) for the estimation of nonspecific binding. The method was that of Leysen et al. (17), except that membranes (260–320 μg protein/ml) were incubated in a total volume of 1 ml of 50 mM Tris-HCl, pH 7.7 (instead of 4.4 ml) (6). Saturation binding curves, with eight concentrations of radioligand ranging from 0.05 to 4 nM, were performed in duplicate from frontal cortices pooled from three rats. Frontal cortices from lean and obese rats were always assayed in parallel (nine rats/group). The B_{max} and K_d values were calculated using EBDA-LIGAND computerized program (20). Protein concentrations were estimated by a Bio-Rad protein assay using bovine γ-globulin as a standard (3).

Statistics

All data are expressed as mean ± SEM. Behavioral and biochemical data were analyzed by means of two-way analyses of variance (ANOVA) with (behavioral data) or without (biochemical data) a repeated factor (number of treatments); if significant, these analyses were followed by Tukey's comparison test.

RESULTS

The head shake responses to acutely administered DOI were decreased by the obesity syndrome, $F(1, 18) = 43.2$, $p < 0.0001$, and by DOI pretreatment, $F(1, 18) = 120.2$, $p < 0.0001$ (Table 1). There was also a significant phenotype × number of DOI injections interaction, $F(1, 18) = 32.4$, $p < 0.0001$, that was due to a decreased head shake response to

TABLE 1

HEAD SHAKE, FOOD INTAKE, AND BODY WEIGHT RESPONSES TO ACUTE OR REPEATED TREATMENT WITH DOI IN LEAN AND OBESE ZUCKER RATS

Treatment	Lean Rats (n = 10)	Obese Rats (n = 10)
Head shakes in 30 min		
DOI × 1	18.81 ± 1.67	6.05 ± 1.21*
DOI × 3	1.18 ± 0.31†	0.39 ± 0.16†
Food intakes during the light phase (g/100 g of body weight)		
Saline × 1	1.28 ± 0.09	1.51 ± 0.07
Saline × 3	1.58 ± 0.22	1.60 ± 0.14
DOI × 1	0.53 ± 0.15	0.57 ± 0.13
DOI × 3	1.60 ± 0.16†	1.41 ± 0.13†
Body weight decreases during the light phase (g/100 g of body weight)		
Saline × 1	1.40 ± 0.25	1.50 ± 0.14
Saline × 3	1.58 ± 0.16	1.62 ± 0.18
DOI × 1	2.39 ± 0.26	2.67 ± 0.19
DOI × 3	1.64 ± 0.26†	1.76 ± 0.18†

Values are given as mean ± SEM. Saline or DOI (2 mg/kg, SC) was administered daily at 0900 and 1900 h (last treatment at 0900 h).

*$p < 0.01$ for the difference between lean and obese animals.

†$p < 0.01$ for the difference between acute and repeated DOI administration.

TABLE 2

EFFECTS OF REPEATED TREATMENT WITH DOI ON [^{3}H]KETANSERIN BINDING IN THE FRONTAL CORTEX OF LEAN AND OBESE ZUCKER RATS

	Lean Rats (n = 9)		Obese Rats (n = 9)	
	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)
Saline × 3	226 ± 27	0.40 ± 0.03	217 ± 24	0.42 ± 0.08
DOI × 3	100 ± 10*	0.39 ± 0.02	62 ± 36*	0.40 ± 0.02

Values are given as mean ± SEM. Saline or DOI (2 mg/kg, SC) was administered daily at 0900 and 1900 h (last treatment at 0900 h). Saturation binding curves with eight concentrations of radioligand were performed in duplicate from three rats sacrificed after a 24-h wash-out period.

*$p < 0.01$ for the effects of DOI.

DOI administration in acutely (but not in repeatedly) treated obese rats (Table 1).

ANOVA revealed that the hypophagic effect of DOI [$F(1, 16) = 22.9$, $p = 0.0002$ for the effect of DOI against saline] was diminished by repeated DOI pretreatment [$F(1, 16) = 13.0$, $p = 0.0024$ for the interaction between drug treatment and treatment number], but not by the rats' phenotype (Table 1). A similar pattern of results was obtained when the effects of acute/repeated DOI administration upon body weights were analyzed (Table 1); thus, DOI administration [$F(1, 36) = 12.8$, $p = 0.001$ for the effect of DOI against saline] and the interaction between drug treatment and treatment number, $F(1, 36) = 13.1$, $p = 0.0009$, but not the phenotype, had significant influences on body weights.

Lastly, [^{3}H]ketanserin binding in frontal cortex was found to be reduced by DOI pretreatment [$F(1, 4) = 29.2$, $p = 0.0006$, compared to saline pretreatment], but not by the rats' phenotype (Table 2).

DISCUSSION

The aim of this study was to analyze whether obesity-related hypercorticism alters some functional (head shake, hypophagia) and biochemical (cortical [^{3}H]ketanserin binding) responses to the acute/repeated stimulation of 5-HT$_{2A}$ receptors. It was first observed that the head shake response to the acute injection of DOI was decreased in obese rats compared to lean animals. Actually, this result did not parallel results showing that hypercorticism elicited by repeated glucocorticoid administration increases the number of body shakes elicited by DOI (15). Before drawing any conclusion, it will be necessary to assess the real impact of hypercorticism (by means of prior adrenalectomy) in the aforementioned alteration in 5-HT$_{2A}$ receptor-mediated head shakes in obese rats.

The results mentioned above suggest that genetic obesity could be associated with decreased 5-HT$_{2A}$ receptor function (at least that mediating head shakes). Nevertheless, the possibility that such a decrease finds its origin at some point distal from the receptor cannot be excluded. Moreover, because obese rats display phenotypic differences (hypercorticism, fat depots) that may alter DOI metabolism and/or bioavailability of DOI, it may be that obese rats displayed fewer head shakes than their lean littermates because fewer molecules of DOI reached their targets. Although only the measurement of plasma/brain DOI levels in lean and obese rats would provide an answer, it is noteworthy that DOI, which is highly lipophilic, was administered on the basis of the rats' body weight (each lean and obese rat was given 600 and 800 μg of DOI, respectively) although the brain weight of the obese rat is slightly (but significantly) decreased (2).

The hypophagic response to the acute administration of DOI [i.e., a behavior thought to be mediated by 5-HT$_{2A}$ receptors (21)] was of identical amplitude in lean and obese rats; logically, this was also true for DOI-elicited decrease in body weight. On the basis of the recent suggestion that 5-HT$_{2C}$ receptors [previously called 5-HT$_{1C}$, see (11)] may also mediate the anorectic effect of DOI (1), putative alterations in 5-HT$_{2C}$ receptors could, at first glance, interact within our paradigm. This possibility may be rejected because the anorectic effect of trifluoromethylphenylpiperazine, which is predominantly mediated by 5-HT$_{2C}$ receptors (13), is of similar intensity in lean and obese Zucker rats (14). Taken together, the results mentioned above suggest that although genetic obesity may be associated with decreased 5-HT$_{2A}$ receptor-mediated functions (e.g., head shakes), this pattern of change may not apply to all 5-HT$_{2A}$ receptor populations (e.g., that mediating hypophagia). However, because (i) feeding data were collected during a time when baseline levels were low, and (ii) short-term anorectic effects of DOI could not be monitored in this study, confirmation of the aforementioned suggestion strictly depends on future experiments aimed at measuring the dose-dependent effects of DOI on head shakes and short-term feeding.

Binding to cortical 5-HT$_{2A}$ receptors [i.e., a population of receptors often taken as an index for the study of centrally located 5-HT$_{2A}$ receptors, see (16)] was not affected in obese rats compared to that of lean rats. This result suggests that hypercorticism does not affect cortical 5-HT$_{2A}$ receptors, a result in line with that of Kendall et al. (12) but opposed to that of Kuroda et al. (15). Because cortical 5-HT$_{2A}$ receptors do not mediate head shakes (18), it is unknown whether the decrease in the head shake response measured in the obese rats is due to [or independent from, see (8)] a decreased density of the 5-HT$_{2A}$ receptors that mediate this behavior.

Rat brain 5-HT$_{2A}$ receptors downregulate rapidly upon repeated stimulation (16). Glucocorticoids are not endowed with a permissive action upon such a regulation inasmuch as adrenalectomy does not affect the extents to which cortical 5-HT$_{2A}$ receptor binding and 5-HT$_{2A}$ receptor-mediated head shakes are diminished by repeated DOI administration (6). However, whether hypercorticism (in intact rats) has an effect upon 5-HT$_{2A}$ receptor downregulation is unknown. In the present study, repeated treatment with DOI decreased in a phenotype-independent manner (i) the number of cortical [^{3}H]ketanserin binding sites, (ii) the number of head shakes elicited by the acute administration of DOI, and (iii) DOI-elicited hypophagia. The results obtained indicate that 5-HT$_{2A}$ receptor downregulation is not affected in the obese rat, thereby suggesting that obesity-related hypercorticism does not alter such a downregulation.

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