Neurotensin Decreases With Fasting in the Ventromedian Nucleus of Obese Zucker Rats

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Neurotensin (NT) inhibits food intake when injected either in brain ventricles or in hypothalamic nuclei such as the ventromedian nucleus (VMN). NT concentrations are lower in obese than in lean Zucker rats in several hypothalamic nuclei, including the VMN. In this experiment, we studied the influence of the feeding state on NT concentrations in different brain areas of 10-week-old lean (n = 27) and obese (n = 27) Zucker rats that were fasted for 48 hours and then refed for 6 hours. NT level was measured in the microdissected areas by radioimmunoassay. Obese rats ingested approximately 50% more food than lean rats in the ad libitum (ad lib) condition ($P < .001$) and 12% more during the refeeding time (NS). NT concentrations in the median eminence (ME) were 50% lower in obese than in lean rats ($P < .001$). This decrease could be related to a 20% decrease in the arcuate nucleus (ARC) of the obese rats ($P < .04$). NT concentrations in the ME and ARC, which are important for the control of pituitary hormone secretion by NT, were not changed by the feeding state in both genotypes. NT varied with the feeding state in the VMN only ($P < .04$). Concentrations were 45% lower in fasted (FD) obese rats than in ad lib or refed (RF) obese rats (1.09 ± 0.25 ng/mg protein *v* 1.98 ± 0.36 ad lib and 1.62 ± 0.11 RF, $P < .05$). They remained unchanged in lean rats. NT variations in the VMN of obese rats could contribute synergistically with other neuropeptides to the abnormal feeding behavior of these rats.

NEUROTENSIN (NT) is a tridecapeptide present both in the central nervous system[1] and in the gastrointestinal tract.[2] It inhibits food intake when injected in different brain areas, but not when given either intraperitoneally or intravenously (see Beck[3] for review). Brain areas responding to NT injection include specific nuclei involved in the regulation of feeding behavior such as the ventromedian (VMN) or paraventricular (PVN) nuclei, but not the lateral hypothalamus (LH).[4-7]

In the first two nuclei, NT content was significantly less in the Zucker fatty rat, a genetic model of obesity with a well-established hyperphagia.[8] We proposed that NT could act with other neuropeptides in the dysregulation of feeding behavior in this strain of rat.[9,10] However, to our knowledge, there are few data on the regulation of hypothalamic NT by the feeding state either in normal rats or in obese animals. Oku et al[11] showed that starvation for 1 or 7 days does not modify hypothalamic NT in either lean or obese (ob/ob) mice. But results in this study were obtained in whole hypothalamus. It is therefore possible that variations in specific hypothalamic nuclei could be masked. Such a situation is not uncommon, since we have recently shown that fat content in the diet influences NT concentrations in the hypothalamus.[12] The variations are localized in the PVN and in the LH, whereas no modifications are observed in other hypothalamic nuclei such as the arcuate (ARC), dorsomedian (DMN), and suprachiasmatic nuclei.[12]

So, to characterize further the effect of fasting and refeeding on central NT, we measured NT concentrations in several microdissected brain nuclei in lean and obese Zucker rats either fasted for 2 days or refed for 6 hours.

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MATERIALS AND METHODS

Animals

Ten-week-old lean Fa/Fa (n = 27) and obese fa/fa (n = 27) Zucker rats bred in our laboratory were housed in individual wire cages in a temperature-controlled room with an automatic 12-hour light-dark cycle with lights on at 2 PM. They were fed a standard chow diet (U.A.R. A04, Villemoisson sur Orge, France) ad libitum. After 15 days of habituation to these conditions, lean and obese rats were randomly distributed into three groups of nine animals. The first group continued to be fed on the same diet ad libitum (ad lib), the second was food-deprived (FD) for 48 hours, and the third was food-deprived for 48 hours and refed (RF) for 6 hours. The duration of refeeding was chosen to determine if the ingestion of a large quantity of food during a limited period of time can influence NT concentrations. Tap water was always available to all rats ad libitum. All rats were killed by decapitation at the same time, ie, at the beginning of the light period. Body weight and food intake were measured during the experiment. Fasting and refeeding effects were assessed by measurement of plasma glucose and β-hydroxybutyrate (OHB) concentrations.

Samplings

Trunk blood was sampled in ice-cooled tubes containing EDTA and aprotinin (Iniprol; Laboratoires Choay, Paris, France).

The brains were quickly removed, frozen on dry ice, and stored at −80°C. Serial sections of 300 μm were cut, and discrete hypothalamic and extrahypothalamic areas were microdissected. Landmarks for this dissection were derived from a standard atlas of the rat brain.[13] The following areas were sampled: PVN, DMN, VMN, ARC, supraoptic nucleus (SON), median eminence (ME), and LH. The bilateral tissue samples were placed in 500 μL of a solution of 0.2N HCl aprotinin-EDTA. Tissue samples were homogenized by sonication, and an aliquot was taken for protein analysis.[14] The remainder of the homogenate was centrifuged at 2,500 × *g* for 30 minutes at 4°C, and the supernatant was stored at −40°C until assay.

Assays

Plasma glucose and OHB concentrations were measured with classic enzymatic procedures using commercial kits (Boehringer Mannheim, Meylan, France, for plasma glucose, and Sigma Diagnostics, La Verpillière, France, for OHB).

NT assay was performed on the neutralized extract with a specific radioimmunoassay developed in our laboratory and previously described.[8] Briefly, standard bovine NT 1-13 (Calbiochem, La Jolla, CA) or samples were incubated with specific NT antiserum and ^{125}I-labeled NT (IM 163; Amersham, Les Ulis, France) for 48 hours, including a 24-hour preincubation period without labeled NT. Bound and free fractions were separated by addition of charcoal-dextran solution. Radioactivity of the bound fraction was measured with a gamma counter coupled to a microcomputer (MDA 312 system; Kontron, Velizy, France) for plotting the standard curve and calculation of the results. For this experiment, maximal binding was 40.9% ± 1.8% and nonspecific binding averaged 2.9% ± 0.7%. All tissue extracts were measured in duplicate.

Statistical Analysis

Results are presented as the mean ± SEM. They were compared by two-way ANOVA for main effects (treatment × genotype) and adequate ANOVA and multiple-range (Fisher protected least significant difference) tests. A probability of less than 5% (two-tailed) was considered significant.

RESULTS

Body Weight and Food Intake

Initial body weights among the three lean groups or among the three obese groups were not significantly different, but obese rats were significantly heavier than lean rats, with overweight reaching 60% (353.4 ± 5.1 *v* 220.4 ± 3.2 g, $P < .001$).

During food deprivation, both lean and obese rats regularly lose weight (~12% to 15% of initial body weight, $P < .001$). At the end of the fasting period, the weight loss of lean rats was significantly less than that of obese rats (32.2 ± 1.6 *v* 42.0 ± 1.2 g, $P < .001$). RF obese rats gained significantly more weight than lean rats (22.3 ± 0.8 *v* 19.3 ± 0.9 g, $P < .05$).

Ad lib obese rats ate approximately 50% more food than lean rats during the 48 hours of the experiment (55.9 ± 1.8 *v* 36.3 ± 0.8 g, $P < .001$). During the 6 hours of refeeding, food intake of both groups did not significantly differ (11.8 ± 0.4 g obese *v* 10.5 ± 0.8 g lean).

Plasma Assays

Food deprivation induced a decrease in plasma glucose and an increase in OHB in both lean and obese rats ($P < .001$; Fig 1). Refeeding induced increases of plasma glucose over the ad lib values in lean and obese rats and restored OHB levels in the obese group only.

Brain Assays

NT concentrations in the different microdissected nuclei are shown in Figs 2, 3, and 4. There was a significant effect of genotype in the ARC and ME (Fig 2), with significantly lower concentrations in both areas in obese rats than in lean rats ($P = .001$ in the ME and $P = .04$ in the ARC). Treatment did not affect these values ($P = .46$ in the ARC and $P = .56$ in the ME). Differences were markedly greater in the ME than in the ARC, since decreases in NT concentrations between lean and obese rats were approximately 50% in the ME and 20% in the ARC.

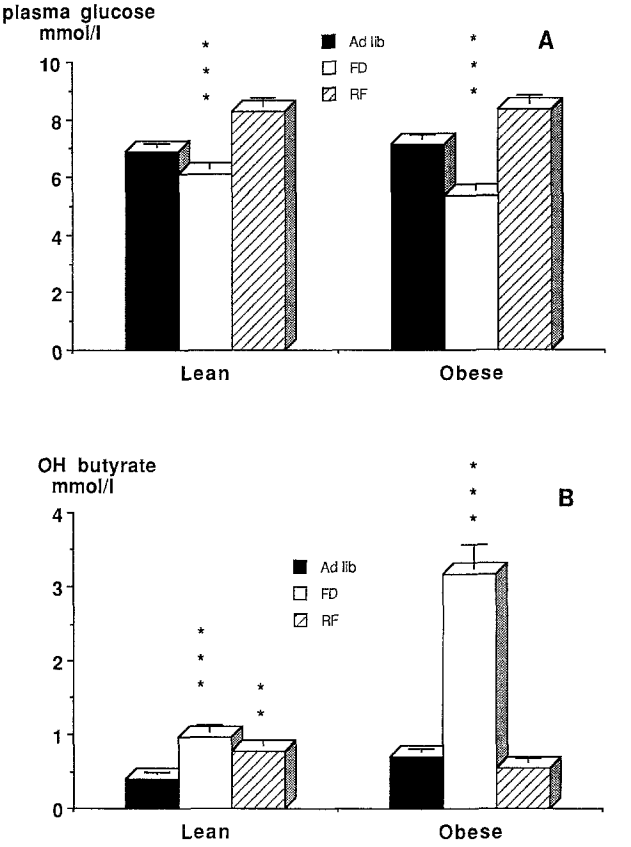

Fig 1. (A) Plasma glucose (mean ± SEM, mmol/L) and (B) OHB (mmol/L) concentrations in lean and obese ad lib, FD, or RF Zucker rats. ***$P < .001$, **$P < .01$: lean *v* obese rats.

In the LH and DMN (Fig 3), there was also a significant effect of genotype ($P = .01$ in the LH and $P = .03$ in the DMN), but no effect of treatment. Opposite variations were noted in these areas. NT concentrations in the LH were 10% to 30% greater in obese than in lean rats, with a significant effect during refeeding in obese rats ($P < .01$). In the DMN, they were 15% less in obese than in lean rats.

In the VMN, there was an effect of treatment only ($P = .04$; Fig 4). Food deprivation induced a decrease of

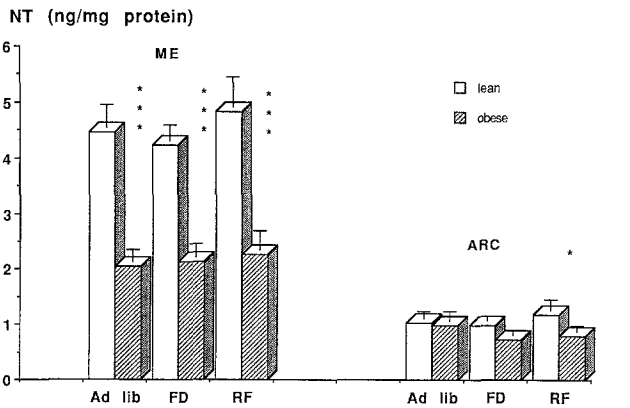

Fig 2. NT (ng/mg protein, mean ± SEM) in the ARC and ME of lean and obese ad lib, FD, or RF Zucker rats. ***$P < .001$, *$P < .05$: lean *v* obese rats.

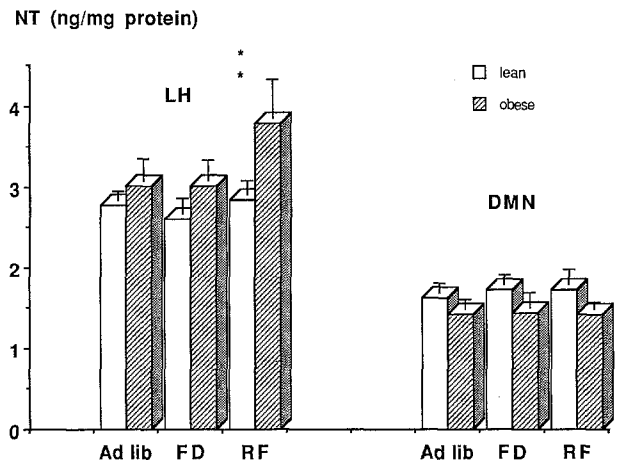

Fig 3. NT (ng/mg protein, mean ± SEM) in the DMN and LH of lean and obese ad lib, FD, or RF Zucker rats. **$P < .01$, lean *v* obese rats.

approximately 45% in NT concentrations in obese rats as compared with ad lib levels ($P < .05$).

Treatment and genotype did not modify NT concentrations in the PVN, SON, or suprachiasmatic nucleus (Table 1).

DISCUSSION

In this experiment, we studied the effect of food deprivation and refeeding on hypothalamic NT concentrations in lean and obese rats. The classic effects of food deprivation (increase in ketone bodies and decrease in plasma glucose) were observed.

NT concentrations varied in several hypothalamic areas. As in a previous study,[8] they were lower in obese than in lean rats in the DMN, ARC, and ME. In the latter structure, the difference was particularly important (50%); it was observed whatever the feeding state. It was related to the lower levels measured in the ARC. Indeed, NT immunoreactivity in the ME is found in fibers,[15] and the source of this immunoreactivity is for the greatest part NT neurons of the ARC.[16,17] Since NT is involved in the control of anterior pituitary hormone secretion,[18,19] the diminution in the ME could be of biologic importance. Plasma basal concentrations of growth hormone are diminished in the obese rat.[20] NT could play a role in this decrease, since NT neurons in the ARC projecting to the ME co-express growth hormone–releasing factor[21] and NT induces important elevations of growth hormone through hypothalamic mechanisms.[19]

In the LH, NT concentrations were among the highest measured in the different microdissected areas, with a range of values between 3 and 4 ng/mg protein. In contrast to the ME, NT levels were enhanced in the LH of the obese rat. This was particularly noted during the refeeding time. The LH is one of the first two areas thought to be important in the regulation of feeding behavior. Lesion of the LH induces aphagia in rats.[22] More recent data indicate that it is also involved when rats ingest a diet rich either in carbohydrates or in fat.[23,24] NT concentrations in this area are modulated by the lipid content of the diet.[24] Yet NT injection in the LH does not modify food intake.[4] Actually, we therefore have no clear explanation for the role of the NT increase in the obese rat after refeeding. Since this area is rich in fibers, it is necessary first to determine the origin of these fibers before further speculating on the role of NT in these conditions.

The VMN is the other classic area involved in the regulation of feeding behavior. Its lesion induces hyperphagia and obesity in rats.[25] It was the only area where the feeding state influenced NT concentrations. An important 50% NT decrease was observed in the obese rat only, after 48 hours of fasting. NT is biologically active in this nucleus through binding sites identified by autoradiography in both lean and obese rats.[26] It can diminish by approximately 50% the food quantity ingested after food deprivation when it is directly injected into the nucleus.[4] A greater decrease in NT could therefore induce a greater feeding response in the obese rat when the food is again available after a period of deprivation. A 12% increase was indeed measured, but it was not significant. Yet intakes during the 6 hours of

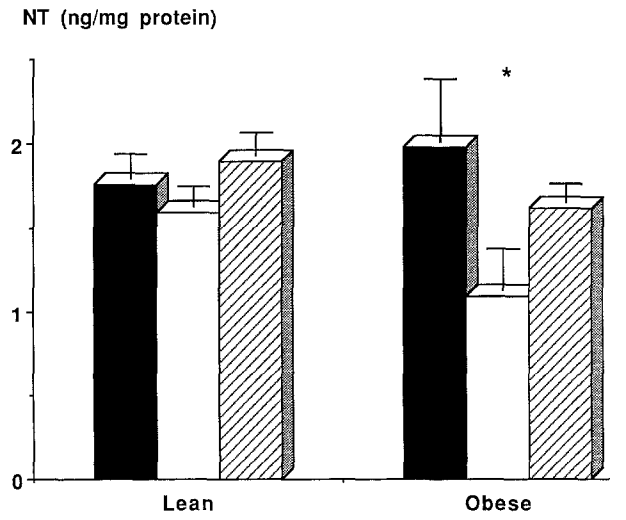

Fig 4. NT (ng/mg protein, mean ± SEM) in the VMN of lean and obese ad lib (■), FD (□), RF (▨) Zucker rats. *$P < .05$, lean *v* obese rats.

Table 1. NT Concentration (mean ± SEM, ng/mg protein) in the SCN, SON, and PVN of Lean and Obese Zucker Rats in Different Feeding Conditions

	Lean			Obese		
Site	Ad Lib	FD	RF	Ad Lib	FD	RF
SCN	0.93 ± 0.08	1.00 ± 0.05	0.94 ± 0.15	0.78 ± 0.13	0.89 ± 0.12	0.92 ± 0.08
SON	1.70 ± 0.10	2.00 ± 0.20	2.39 ± 0.22	2.01 ± 0.16	1.91 ± 0.24	1.98 ± 0.14
PVN	1.12 ± 0.12	1.08 ± 0.15	1.12 ± 0.12	1.15 ± 0.11	1.08 ± 0.15	1.08 ± 0.10

Abbreviation: SCN, suprachiasmatic nucleus.

refeeding were maximal in obese, as well as in lean, rats. They corresponded to half of the daily intakes. Stomach size and gastric emptying could be limiting factors in this case.

In conclusion, the feeding state influenced hypothalamic NT in a specific and important area involved in the regulation of feeding behavior. The decrease in the VMN of fasted obese rats could contribute to their abnormal feeding behavior synergistically with elevated neuropeptide Y levels in the PVN.[27] The latter also reflects a state of food deprivation.[28] The involvement of several neuropeptides and different brain areas shows the complexity of this dysregulation.

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