Physiological regulation of hypothalamic neuropeptide Y release in lean and obese rats

A. STRICKER-KRONGRAD, R. KOZAK, C. BURLET, J. P. NICOLAS, AND B. BECK
Institut National de la Santé et de la Recherche Médicale U308 Mécanismes de Régulation du Comportement Alimentaire, 54000 Nancy, France

Stricker-Krongrad, A., R. Kozak, C. Burlet, J. P. Nicolas, and B. Beck. Physiological regulation of hypothalamic neuropeptide Y release in lean and obese rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R2112–R2116, 1997.—The paraventricular nucleus (PVN) of the hypothalamus is an important site for the regulation of feeding behavior. Neuropeptide Y (NPY) injected into this nucleus strongly stimulates food intake. In the current study, we measured NPY release in the PVN of unrestrained rats through the push-pull technique. The rats were placed in their habitual environment and conditions of life. NPY release was augmented by >40% (P < 0.01) in Long-Evans rats deprived of food for 12 h. It returned to the baseline as measured in ad libitum-fed rats 90 min after food access. Its stimulation by 55 mM KCl in fasted animals indicated that the whole stock of NPY was not used during a short fast. During the light-dark transition, when feeding behavior is initiated, NPY release in lean Zucker rats showed a peak 20 min after lights off and then declined. It corresponded well with the first feeding episodes. In the obese Zucker rats, this peak was absent. NPY release was totally anarchic but at a high level. The feeding behavior of the obese rats was not as time delimited as in the lean rats. This study performed in very physiological conditions therefore indicates that NPY release could drive feeding behavior in the normal life. Its dysregulation in obese rats could participate in overeating and absence of feeding rhythm measured in these rats and speed up the development of their obesity.

MATERIALS AND METHODS

Experiment 1. Male Long-Evans (CERJ, Le Genest-Saint-Ise, France) weighing 250–300 g were used for this study. They were housed in plastic cages in the vivarium with a 12:12-h light-dark cycle (lights on 0700–1900). They were fed on a standard laboratory diet (A04, UAR, Villemoisson sur Orge, France) ad libitum and had tap water to drink. After at least 2 wk of habituation, the rats were anesthetized by intraperitoneal injection of chloral hydrate-ketamine (Ketalar, Parke-Davis, France; 150 mg/kg body wt). They were stereotaxically implanted with a push-pull cannula (external cannula 500 µm, internal cannula 100 µm with 250-µm protusion) with a guide cannula placed above the right PVN. After 1 wk of recovery, they were perfused with artificial cerebrospinal fluid (CSF) in our climatized push-pull room for 8 h in the light period. The rate of perfusion was 13 µl/min. The perfusion generally started 1 h after lights on. Thirty-minute samples were collected into polypropylene tubes containing 10 µl of aprotinin (Iniprol, Laboratoires Choay, France) and kept on ice. The samples were lyophilized and stored at −40°C before assay for NPY.

The rats were divided into two groups. The first group was perfused in standard free-moving conditions with food and water available during the entire session. The second group

THE HYPOTHALAMIC paraventricular nucleus (PVN) is an important site where autonomic and neuroendocrine regulatory information is integrated (33). It is involved in the regulation of feeding behavior (16, 20) and contains numerous peptides that mediate food intake (23). Among them, neuropeptide Y (NPY) is found in abundance in fibers (14). These fibers mainly originate in the arcuate nucleus (ARC) (2) and in the brain stem (27). The PVN plays a critical role in the effect of NPY on feeding behavior. This has been demonstrated by several approaches. First, NPY injection in this nucleus strongly stimulates food intake (12, 29). Sensitivity to injection of exogenous NPY is enhanced when the NPY concentrations in the PVN are diminished either by the neurotoxic effect of monosodium glutamate on the arcuate neurons (31) or by transections of the neural connections between the brain stem and PVN (24). On the other hand, the orexigenic effects of NPY can be blocked by direct injection of antibodies to NPY into the PVN (28). Second, fasting and refeeding modulate NPY concentrations in the PVN (6, 25). These concentrations also vary according to the light-dark cycle. A peak is observed at the beginning of the dark phase (17), and it is well known that this particular period is characterized by the ingestion of the first meals by the rat. Finally, by use of push-pull or microdialysis techniques, several researchers have shown that NPY is released in the PVN in the basal state (19, 30) and in food-deprived animals (19). This release can be stimulated in vivo by a potassium-induced depolarization (30).

NPY in the PVN is also involved in abnormal feeding behavior as observed in the anorexic tumor-bearing rats and in the hyperphagic obese Zucker rats. Diminished and augmented concentrations are respectively measured in the two models (4, 11, 22). The hyperphagia in obese Zucker rats is also characterized by an absence of regulation by the feeding state and a decreased sensitivity to NPY injection (5, 32). The dynamic release of NPY is diminished in tumor-bearing rats (11), but in hyperphagic Zucker rats, contradictory results were reported. In vitro, basal NPY efflux from the PVN of obese rats was not different from that measured in lean rats (18). In vivo, however, release was significantly augmented (13).

Therefore, in view of this information, the goal of the present experiment was to ascertain the role of NPY in the PVN in the normal and perturbed feeding behavior in very physiological conditions. For this purpose, we first confirmed that NPY is released in greater quantity after a short fast, and we then described the total anarchy in the NPY release during the light-dark transition in the obese Zucker rat.
was food deprived for 12 h, i.e., corresponding to the dark period preceding the perfusion period. Water remained available. Ninety minutes after the beginning of the perfusion session, the rats were given access to food. A subgroup of this second group (n = 3) was perfused with hyperosmotic CSF (55 mM KCl) for 30 min starting 3 h after the beginning of the session and then reperfused with normal CSF until the end of the session.

Animal behaviors were recorded during the entire perfusion time by one experimenter. Each of the mutually nonexclusive following behaviors was recorded: moving, sleeping, resting, and eating.

At the end of the perfusion periods, the rats were killed by decapitation, and brains were removed for histological control. Animals with misplacement of the cannula or tissue damage were discarded from the study.

Experiment 2. Six-month-old lean (Fa/−) and obese (fa/fa) Zucker rats born in our laboratory were used. Obese rats were significantly heavier than the lean ones (542.0 ± 5.0 vs. 390.5 ± 5.6 g; P < 0.0001). They were adapted to an inverse light-dark cycle (lights off at 1500). They were fed on a standard laboratory diet (A04, UAR) ad libitum and had tap water to drink.

They were cannulated as previously described for the Long-Evans rats. They were allowed at least 1 wk of recovery. The day before perfusion, they were transferred from the vivarium to our temperature-regulated push-pull room. On the perfusion day, the push-pull cannula was installed 3 h before lights off. This was followed by a period of equilibration of 2 h with perfusion of artificial CSF. Samples were then obtained every 20 min for a period of 3 h starting 1 h before lights off. The perfusion rate was the same as for the Long-Evans rats (13 µl/min). When the lights went off, a red light went on to allow the experimenter to control the perfusion and to write down the animal behavior. Previous measurement showed that this does not modify the feeding behavior of the rats. Cumulative time spent eating was measured during each sampling period.

As for the Long-Evans, the Zucker rats were decapitated for the control of the cannula placement. The main reason for discarding animals was rupture of the third ventricle indicated during perfusion by an unbalanced flow (too much efflux) and laterality. Eleven lean and nine obese rats were finally used for NPY measurement and data analysis.

Trunk blood was sampled for the determination of the plasma glucose and triglycerides to characterize the lean Fa/− and obese fa/ fa rats.

Assays. Plasma glucose and triglycerides were measured by enzymatic techniques using commercially available kits (Boehringer-Mannheim, Meylan, France). NPY was measured with a specific radioimmunoassay developed in our laboratory as previously described (4). In the present experiment, maximal binding was 45.0 ± 2.5%. A 50% decrease of the bound activity was obtained with a concentration of 0.41 ± 0.05 ng/ml of NPY. Assay sensitivity was 5 pg/tube. Nonspecific binding was 6.0 ± 0.4%. For the assay, the lyophilized samples were reconstituted with 0.04 M phosphate buffer (pH 7.4) containing bovine serum albumin (fraction V, Sigma Chemicals, La Verpillière, France), aprotinin (4,000 IU/ml), and sodium azide (Merck, Darmstadt, Germany).

Statistical analysis. Results were compared by two-way analysis of variance and Friedman's and Student's t-tests. Only P < 0.05 was considered significant.
is measured immediately after the food access in the food-deprived rats. Before food access, the food-deprived rats are characterized by an augmentation in the time spent moving (P < 0.05). Potassium infusion induced a new episode of food intake (Fig. 3).

Experiment 2. At the end of the experiment, all rats had gained weight. Final body weight of lean rats was 412.3 ± 4.5 g, and that of the obese rats was 627.8 ± 26.5 g. Obese rats were hyperglycemic [7.80 ± 0.26 (fa/fa) vs. 6.68 ± 0.26 (Fa/−) mM; P < 0.01] and hypertriglyceridemic [3.31 ± 0.26 (fa/fa) vs. 0.56 ± 0.03 (Fa/−) mM; P < 0.001].

Feeding behavior of the Zucker rats is shown in Fig. 4. In the lean rats, there were two clear-cut meals (P = 0.003) observed 20 and 100 min after lights off. The profile of the feeding behavior in the obese rats looked like that of the lean rats, but due the large variations observed between animals, typical meals could not be detected (P = 0.55). Obese rats had a tendency to spend more time for eating.

NPY release in both groups is shown in Fig. 5. In lean rats, NPY release progressively increased during the last hour of the light period and peaked 20 min after the beginning of the dark period (11.5 ± 1.4 vs. 6.2 ± 1.0 pg/tube; P < 0.013). It then regularly decreased until the end of the perfusion period. This phenomenon was not observed in the obese rats, and the profile was very irregular without any significant variation during the entire perfusion. However, the levels of release were augmented at least by 50% at the beginning (P = 0.012) and end (P = 0.034) of the perfusion period in obese compared with lean rats. These levels were equivalent to the peak of release in the lean rats.

DISCUSSION

In this study, we investigated the dynamic release of NPY in the hypothalamus of rats through the push-pull technique. NPY is actually considered to be the most potent stimulator of food intake and therefore to play a major role in the regulation of feeding behavior (reviewed in Ref. 29). Most of the information concerning the role and regulation of NPY arises from measurements of its brain concentrations in different feeding conditions. It was thus shown that its concentration increases in the ARC and PVN in food-deprived animals. It is normalized by food ingestion (6, 25) and can be modulated by diet composition at short and long term (7, 8). It presents a nycthemeral rhythm in the ARC and PVN (17). Its increased levels could also participate in the development of hyperphagia and obesity in the Zucker fa/fa rat (3, 4, 9). Measurements of NPY mRNA expression in the ARC are generally in good agreement with the peptide content (26). However, these two measures of content and expression only reflect a timely situation and cannot give the
entire picture of what really happens during feeding. The measurement of the release will give an idea of the functional variations of NPY because it can be measured in undisturbed, free-moving rats placed in their habitual environment. The PVN was the hypothalamic site chosen for the placement of the push-pull cannula because of its particular sensitivity to NPY injection and its general involvement in numerous feeding conditions (reviewed in Ref. 29). Several previous studies have already shown that NPY is released in the basal fed state in this nucleus (19, 30). We confirmed these data in the present study. Moreover, in our rats placed in a situation of a light fast (12 h), we showed that this basal liberation is augmented by >40% in the food-deprived animals. This high release is associated with an increased activity of the animals. As latency to eat in satiated animals is decreased after artificial augmentation of hypothalamic NPY by exogenous injection (12, 21, 29), it might induce the search for food and motivation to eat (15). This result is in good agreement with those obtained after a much longer fast of 3 days (19). We noted that when food was available, the secretion returned to ad libitum levels within 90 min. This return was delayed to 3 h after a 20-h fast in rats on scheduled feeding regimen and to 24 h in 3-day-fasted rats (19). It therefore appears that the level of secretion is proportional to the duration of fast and can adapt itself to the energy need. In the case of the 12-h fast, all reserves of NPY were not mobilized, since it was still possible to stimulate the release through hyperosmotic potassium. This might confirm this adaptive mechanism to the importance of food deprivation, but the effects of KCl are not specific. The KCl-stimulated NPY release might also derive from a pool not related to food intake, having its origin in the brain stem, and the associated feeding behavior may be related to the release of other neuromediators. Further experiments are needed to clarify this point.

In Zucker rats, we used another strategy that is even more relevant to the normal behavior of the rats. We measured the NPY release during the light-dark transition. This period is well known for its activation of feeding behavior in the rat. It is characterized by a peak of NPY concentration in the ARC as well as the PVN (17). The basal NPY release in Zucker rats was lower than that of the Long-Evans rats. This was probably related to the animals themselves by combining a strain effect with an age effect, since our Zucker rats were older than the Long-Evans rats.

In the lean Zucker rat, we showed a progressive increase in the liberation of NPY, which reached its maximum 20 min after the lights were off. This increase was associated in time with the first meal that occurred very rapidly after lights off. NPY release declined slowly after this first period of food ingestion and increased slightly when the second meal was consumed. These results agree with those obtained in the fasting experiment. They mimic the situation observed with punctual measurements of the tissue concentration and argue for a role of NPY in triggering feeding behavior in early dark. In the obese Zucker rats, the release of NPY was completely different. It did not vary significantly during the whole experimental period even if it presented a jagged profile. However, the mean level of secretion corresponded to the peak measured in lean animals and was significantly higher than the basal state in the lean animals. Food ingestion did not affect the release in the obese rats. The feeding behavior profile looked like that observed in the lean rats, but due to the very large variability between obese animals, it was impossible to have clear-cut meals at the same time. These data reinforce several ideas on the role of NPY in obesity established from variations of concentration and expression. First, the exacerbated release, which is also observed during the light period (13), could continuously stimulate feeding behavior and explain the disappearance of the classical light-dark rhythm of food intake in the obese rat (1, 10). This high release could also participate in the decreased sensitivity to exogenous NPY (32). It corresponds to a maximal synthesis of the peptide in the ARC that cannot be further enhanced by fast (5). Its insensitivity to food ingestion agrees with the absence of effect of refeeding after fasting (5).

In conclusion, this study of the dynamic release of NPY in the PVN sheds light on the physiology of this peptide in unrestrained lean and obese animals. This release is particularly sensitive to the repletion of the energy stores in the lean animals. The sustained anergic NPY release in the Zucker fa/ fa rat is a feature of obesity in this animal model. This type of study, which is much more complicated to perform in animals with spontaneous nocturnal activity, was necessary to understand its regulation well and to identify elements for developing new processes and/or strategies for treating obesity.

**Perspectives**

This study emphasized the role of both an enhanced and dysregulated release of NPY in obese rats. These changes could participate in the modified feeding behavior of these rats and therefore contribute to the development of their overweight. Future strategies for limiting these disorders include the development of drugs that could control the synthesis of this peptide. Research is also necessary to better understand the mechanisms controlling the secretion of this peptide. A well-controlled release would also help in normalizing intake and weight gain. It must also be kept in mind that
each behavior is the result of a balance between neuropeptides or more generally neuromediators. For now, a multitargeted drug strategy, such as that currently used with AIDS, appears essential to fight these disorders.

In the future, the best option would be to prevent the development of these disorders rather than treat them when they are established. This will need additional research as well as a change in the general approach to these problems at both research and political levels.

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Address for reprint requests: B. Beck, INSERM U308, 38 rue Lionnols, 54000 Nancy, France.

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