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Fasting Affects More Markedly Neuropeptide Y Than Monoamines in the Rat Brain

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PAGES, N., M. OROSCO, C. ROUCH, O. YAO, C. JACQUOT AND C. BOHUON. *Fasting affects more markedly neuropeptide Y than monoamines in the rat brain.* PHARMACOL BIOCHEM BEHAV 44(1) 71–75, 1993.—Monoamine turnover and neuropeptide Y (NPY) levels were evaluated in the CNS of 48- and 72-h-fasting adult, male rats in four brain areas: the hypothalamus, cortex, hippocampus, and striatum. In 48-h-fasted rats, NPY levels increased in the cortex and decreased in the striatum. The dopaminergic turnover increased in the hippocampus. The serotonergic turnover decreased in the hippocampus, striatum, and cortex and was still decreased after 72 h of fasting. In 72-h-fasted rats, an overall significant increase of NPY levels was observed except in the striatum, where it decreased significantly. No relationship appeared between NPY and monoamine levels, suggesting that NPY can act independently in feeding behavior and play, in different brain areas, an important role in its regulation.

NPY Catecholamines Serotonin Fasting

NEUROPEPTIDE Y (NPY), a 36 amino acid residue of the pancreatic polypeptide family, is located in large dense-core vesicles of nerve cell bodies and terminals both in the peripheral and central nervous system (1,2,10,14,22,34), in particular in hypothalamic areas that regulate feeding behavior (18). Indeed, one of its physiological functions is to be a potent stimulator of food intake in mice and rats because the injection of NPY into the paraventricular nucleus of the hypothalamus (PVN) or into different central nuclei stimulates drinking and feeding behavior, in particular carbohydrate ingestion, in satiated animals (8,9,20,24–26,30,31). In the rat, its action is further characterized by an indirect effect on pancreatic β-cells because it stimulates insulin release at doses that enhance food intake (23).

NPY has been found to coexist with catecholamines in central and peripheral structures (15,21), suggesting that this peptide interacts with other systems and especially with the adrenergic system (3). Specifically, NPY is found to coexist with norepinephrine (NE) in the PVN (11,29). Extensive work has identified the PVN as a primary site in the mediation of feeding elicited by NE (19). The implication of the remaining monoamines in feeding behavior has been also reported. Destruction of dopaminergic or catecholaminergic fibers can lead to hypophagia (25). Dopamine (DA) infusion can increase or decrease feeding according to the site of injection (18). Finally, various studies suggest that serotonin functions as a satietogen agent (18,26). However, the interrelationships between NPY and monoamines in feeding behavior remain unclear. It seems that, at least in the PVN, NPY can act independently of NE (17). The aim of the present work was to study simultaneously the variations induced by 48 or 72 h of fasting on the physiological levels of NPY and on the turnover of monoamines in four distinct cerebral areas: the hypothalamus, cortex, hippocampus, and striatum.

METHOD

Animals and Treatment

Male Sprague-Dawley rats (IFFA Credo, France) weighing 170–190 g were used. They were kept under a controlled 12 L : 12 D schedule (light from 6:00 a.m.–6:00 p.m.) at 23 ± 1°C and had free access to food and tapwater for 1 week before experimentation. They were assigned at random to one control and two experimental groups (n = 5) and housed at a maximum of three per cage. The experimental groups were deprived of food in the middle of the light period. Then, 48 or 72 h following initiation of food deprivation rats were sacrificed.

Tissue Collection

Animals were decapitated between 12:00 and 2:00 p.m. to eliminate the influence of diurnal variations. Brains were quickly removed and dissected on a chilled plate to separate

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TABLE 1

EFFECT OF FASTING IN RATS ON CEREBRAL NPY LEVELS (pM/g)

Fasting (h)	Brain Area			
	HT	HC	S	CX
0	920 ± 20	473 ± 20	392 ± 35	436 ± 46
48	902 ± 33	474 ± 28	259 ± 30*	557 ± 51†
72	1220 ± 31†‡	618 ± 30§¶	267 ± 22*	561 ± 14†

HT, hypothalamus; HC, hippocampus; S, striatum; CX, cortex.
*$p < 0.05$; †$p < 0.001$, §$p < 0.01$ vs. controls; ‡$p < 0.001$, ¶$p < 0.01$ vs. 48-h-fasting rats.

the hypothalamus, hippocampus, striatum, and cortex, which were then stored at −80°C. After thawing, the brain areas were homogenized in 0.4 N perchloric acid containing 0.1% EDTA, $Na_2S_2O_5$, and cystein for NPY assay. The homogenates were centrifuged (3,500 × g, 15 min, 4°C) and the supernatants were stored at −80°C until monoamine determinations.

Monoamine and NPY Assays

NPY concentrations were measured on brain homogenates by an immunoradiometric assay (IRMA) using two monoclonal antibodies (MAB) (12). The first MAB (NPY 02) was a mouse IgG 1 with a K_d of 5.5×10^{-10} M for the total NPY and recognizing an epitope localized in the 10-15 region of the NPY. The second MAB (NPY 05) was a mouse IgG 2 with a K_d of 2.5×10^{-10} M for the total NPY and recognizing an epitope localized in the 32-36 $CONH_2$ region of the NPY. NPY 02 was used as the uptaker and was coated on polystyrene tubes and NPY 05 was used as the indicator after labeling it with ^{125}I using the iodogen method. The assay was performed by adding 250 μl of the sample to NPY 02-coated tubes followed by the addition of 50 μl of the labeled NPY 05. The tubes were incubated overnight at +4°C. After incubation, the tubes were washed three times with 0.15% Tween-20 in distilled water and counted in a γ-radioactivity counter. Results were expressed as pM/g tissue.

Monoamine and metabolite concentrations in the supernatant of brain were analyzed as previously described (27) using a liquid chromatography technique with an electrochemical detection. NE, DA, dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), 5-hydroxytryptamine (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) were separated and quantified in the brain. Results were expressed as ng/g tissue.

Statistical Analysis

Results were expressed as the mean ± SEM of five determinations. Statistical significance was calculated by analysis of variance (ANOVA). The difference between the different groups was calculated using the multiple-comparisons Dunnett's test.

RESULTS

After starvation, NPY levels were affected differentially according to the cerebral area (Table 1). In the 48-h-fasted rat group, NPY increased, $F(2, 14) = 60.47$, $p < 0.001$, only in the cortex (+24%). The same tendency was observed in the hippocampus but did not reach the level of significance. No changes were observed in the hypothalamus. In the 72-h-fasted rat group, NPY levels were increased in the hypothalamus up to 32%, $F(2, 14) = 38.15$, $p < 0.001$, in the hippocampus up to 30%, $F(2, 14) = 9.99$, $p < 0.01$, and in the cortex up to 28%, $F(2, 14) = 60.47$, $p < 0.001$, as compared to controls. In the hypothalamus, NPY levels after 72 h of fasting were significantly increased when compared to the 48-h-starved rat group, $F(2, 14) = 38.15$, $p < 0.001$. In the striatum, NPY levels decreased significantly after 48 h (−34%) and 72 h (−32%) of fasting, $F(2, 14) = 6.30$, $p < 0.05$.

In any brain area, the noradrenergic levels were not altered (data not shown). The dopaminergic turnover increased only in the hippocampus of the 48-h-fasted rat group and returned to normal values after 72 h of fasting (Table 2). DA and DOPAC levels were significantly increased as compared to controls and to the 72-h-fasted rat group [$F(2, 13) = 32.06$, $p < 0.001$, and $F(2, 13) = 17.47$, $p < 0.001$, respectively]. 3-MT did not vary.

In contrast, the 5-HIAA/5-HT ratio showed an overall tendency to decrease during fasting as a result of two opposite but not always significant variations of the two parameters (Table 3). The serotonin level increased in the striatum, $F(2, 14) = 6.54$, $p < 0.01$, and cortex, $F(2, 14) = 3.94$, $p < 0.05$, in 72-h-starved rats as compared to controls and to the 48-h-fasted rat group. 5-HIAA level decreased in the cortex in both fasted groups, $F(2, 14) = 7.76$, $p < 0.01$. Finally, the 5-HIAA/5-HT ratio was significantly decreased in the hippocampus, $F(2, 13) = 20.95$, $p < 0.001$, striatum, $F(2, 14) = 4.06$, $p < 0.05$, and cortex, $F(2, 14) = 5.40$, $p < 0.05$.

DISCUSSION

The regulation of food intake is an extremely complex process including peripheral and central feeding systems and is far from being completely elucidated. The central feeding system integrates peripheral signals through a cascade of various neu-

TABLE 2

EFFECT OF FASTING ON HIPPOCAMPAL DOPAMINERGIC TURNOVER (ng/g)

Fasting (h)	DA	DOPAC	3-MT
0	69 ± 12	47 ± 7	249 ± 11
48	154 ± 4*†	80 ± 3*‡	274 ± 13
72	87 ± 4	49 ± 3	272 ± 12

*$p < 0.001$ vs. controls, †$p < 0.001$, ‡$p < 0.01$, vs. 72-h-fasting rats.

TABLE 3

EFFECT OF FASTING ON SEROTONERGIC TURNOVER

Fasting (h)	Hypothalamus			Hippocampus			Striatum			Cortex		
	5-HT	5-HIAA	5-HIAA/5-HT	5-HT	5-HIAA	5-HIAA/5-HT	5-HT	5-HIAA	5-HIAA/5-HT	5-HT	5-HIAA	5-HIAA/5-HT
0	402 ± 32	596 ± 19	1.53 ± 0.15	249 ± 9	369 ± 10	1.48 ± 0.03	235 ± 12	645 ± 26	2.80 ± 0.22	239 ± 21	246 ± 14	1.07 ± 0.14
48	384 ± 26	570 ± 27	1.53 ± 0.17	274 ± 9	320 ± 20	1.17 ± 0.05*	237 ± 3	526 ± 38	2.22 ± 0.19†	241 ± 15	183 ± 12‡	0.76 ± 0.04†
72	430 ± 50	556 ± 61	1.30 ± 0.04	272 ± 9	324 ± 19	1.19 ± 0.05*	277 ± 9†§	604 ± 33	2.17 ± 0.05†	290 ± 7†§	209 ± 8†	0.72 ± 0.05†

*$p < 0.001$, †$p < 0.05$, ‡$p < 0.01$, vs. controls; §$p < 0.05$ vs. 48-h-fasting rats.

rotransmitters, involving monoamines and peptides (25). Recent works have shown that NPY has a potent stimulatory effect on feeding. Many studies have focused on the hypothalamus, suggesting a physiological role of NPY in food intake regulation at the PVN level (6,33). The increase in food consumption is linked to an important increase in body weight and body fat when NPY is chronically injected (32). Recently, significant increases in NPY content were described in genetically obese Zucker rats in various nuclei of the hypothalamus involved in the control of food intake, feeding periodicity (16), and energy balance and also in the arcuate mediane eminence, where NPY is produced in large amounts (5). It was also shown that NPY levels rise and fall with the nutritional state of animals in various hypothalamic nuclei (4,6,28). Finally, NPY arises notably before the onset of the active period (16). Conversely, an increase in NPY levels in 72-h-fasting rats has been recently described in the microdissected area of the hypothalamus including the PVN by means of radioimmunoassay (28) and immunochemistry (6). In contrast, there are few findings in the literature on the role of NPY in feeding regulation in other brain areas.

In the present work, we studied the effect of 48- and 72-h starvation on the levels of monoamines and NPY in four distinct central areas: the hypothalamus, cortex, hippocampus, and striatum. In a previous study in mice (unpublished data), we observed a steady-state or even a slight but unsignificant decrease in hypothalamic NPY levels when animals were starved 12–48 h. In the present study, the NPY level was also unchanged in the hypothalamus of 48-h-fasted rats. It may be assumed that an increase, if any, in some nuclei could not be observed in the whole hypothalamus. In contrast, and in agreement with previous studies (6,28), it increased significantly in the hypothalamus (+32%) of 72-h-starved rats, thus suggesting important variations in various hypothalamic nuclei. A significant increase was also observed in the hippocampus after 72 h (+30%) and in the cortex after 48 and 72 h of fasting (+24% and +28%, respectively). However, this increase was not generalized in the brain because a significant decrease in the striatal NPY level by about 30% was observed after 48 and 72 h of starvation. The physiological significance of these changes needs to be investigated.

Except for the striatum, the generalized increase of NPY in the brain could be due either to an increase of NPY synthesis or a decrease of NPY release or metabolism or to both of these mechanisms. It may be hypothetized that, at least in the hypothalamus, an increased synthesis of NPY takes place as a consequence of the decrease of peripheral satiety signals including vagal (cholecystokinin, somatostatin, glucagon) and nonvagal inputs (bombesin, calcitonin).

In contrast, fasting induced little change in cerebral monoamines. NE levels did not vary in any area. According to Kyrkouli et al. (17), who showed that NPY injected into the PVN remains effective in eliciting feeding in the presence of α_2-receptor antagonists and catecholamine synthesis inhibitors, it seems that NPY can act independently of endogenous NE. The dopaminergic turnover was not altered in the cortex although DA utilization has been shown to be increased in the prefrontal cortex after fasting, but this was ascribed to stress (7). The increases in DA and DOPAC observed in the hippocampus after 48-h fasting may also be ascribed to stress. The serotonergic turnover decreased in the hippocampus, striatum, and cortex but not in the hypothalamus, in agreement with other authors (13). This indicates that serotonin, considered a satietogen agent, is slowly released in these areas during fasting. However, the variations in NPY in these regions may be either opposite or parallel to those of 5-HT, indicating a lack of relation between these two systems. Like for NPY, the absence of significant changes in monoamine levels in the hypothalamus could be due to the fact that discrete variations occurring in some nuclei could not be observed in the whole hypothalamus.

In conclusion, it seems that NPY is more sensitive than the monoamines to the stress of feeding. In any brain area, no relationship between NPY and monoamine variations appeared, suggesting that NPY can act independently and may be involved in the regulation of feeding in many cerebral areas.

REFERENCES

1. Allen, J. M.; Bloom, S. R. Neuropeptide Y: A putative neurotransmitter. Neurochem. Int. 8:1–8; 1986.
2. Allen, Y. S.; Adrian, J. E.; Allen, J. M.; Tatemoto, K.; Crow, T. J.; Bloom, S. R.; Polak, J. M. Neuropeptide Y distribution in the rat brain. Science 221:877–879; 1983.
3. Beal, M. F.; Frank, R. C.; Ellison, D. W.; Martin, J. B. The effect of neuropeptide Y on striatal catecholamines. Neurosci. Lett. 71:118–123; 1986.
4. Beck, B.; Burlet, A.; Nicolas, J. P.; Burlet, C. Hypothalamic neuropeptide Y (NPY) in obese Zucker rats: Implications in feeding and sexual behaviors. Physiol. Behavior. 47:449–453; 1990.
5. Beck, B.; Jhanwar-Uniyal, M.; Burlet, A.; Chapleur, M.; Leibowitz, S. F.; Burlet, C. Rapid and localized alterations of neuropeptide Y (NPY) in discrete hypothalamic nuclei with feeding status. Brain Res. 528:245–249; 1990.
6. Calza, L.; Giordino, L.; Battistini, N.; Zanni, M.; Galetti, S.; Protopa, F.; Velardo, A. Increase of neuropeptide Y-like immunoreactivity in the paraventricular nucleus of fasting rats. Neurosci. Lett. 104:99–104; 1989.
7. Carlson, J. N.; Herrick, K. F.; Baird, J. L.; Glick, S. D. Selective enhancement of dopamine utilization in the rat prefrontal cortex by food deprivation. Brain Res. 400:200–203; 1987.
8. Clark, J. T.; Kalra, P. S.; Crowley, W. R.; Kalra, S. P. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology 115:427–429; 1984.
9. Clark, J. T.; Kalra, S. P.; Kalra, P. S. Neuropeptide Y stimulates feeding but inhibits sexual behaviors in male rats. Endocrinology 117:2435–2442; 1985.
10. Emson, P. C.; De Quidt, M. E. A new member of the pancreatic polypeptide family. Trends Neurosci. 7:31–35; 1984.
11. Everitt, B. J.; Hökfelt, T.; Terenius, L.; Tatemoto, K.; Mutt, V.; Goldstein, M. Differential coexistence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neuroscience 11:443–463; 1984.
12. Grouzmann, E.; Comoy, E.; Bohuon, C. Neuropeptide Y concentrations in patients with neuroendocrine tumors. J. Clin. Endocrin. Metab. 68:808–813; 1989.
13. Heffner, T. G.; Vosmer, G.; Seiden, L. S. Time-dependent changes in hypothalamic dopamine metabolism during feeding in the rat. Pharmacol. Biochem. Behav. 20:947–949; 1984.
14. Hendry, S. H.; Jones, E. G.; Emson, P. C. Morphology, distribution, and synaptic relations of somatostatin- and neuropeptide Y-immunoreactive neurons in rat and monkey neocortex. J. Neurosci. 4:2497–2517; 1984.
15. Hokfelt, T.; Lundberg, J. M.; Lagercrantz, H.; Tatemoto, K.; Mutt, V.; Lindberg, J.; Terenius, L.; Everitt, B.; Fuxe, K.; Agnati, L.; Goldstein, M. Occurrence of NPY-like immunoreactivity in catecholamine neurons in the human medulla oblongata. Neurosci. Lett. 36:217–222; 1983.
16. Jhanwar-Uniyal, M.; Beck, B.; Burlet, A.; Leibowitz, S. F. Diur-

nal rhythm of neuropeptide Y-like immunoreactivity in the suprachiasmatic, arcuate and paraventricular nuclei and other hypothalamic sites. Brain Res. 536:331–334; 1990.
17. Kyrkouli, S. E.; Stanley, B. G.; Hutchinson, R.; Seirafi, R. D.; Leibowitz, S. F. Peptide-amine interactions in the hypothalamic paraventricular nucleus: Analysis of galanin and neuropeptide Y in relation to feeding. Brain Res. 521:185–191; 1990.
18. Leibowitz, S. F. Neurochemical systems of the hypothalamus: Control of feeding and drinking behavior and water electrolyte excretion. In: Morgan, P.; Panksepp, J., eds. Handbook of the hypothalamus. vol. 3. New York: Marcel Dekker; 1980:299.
19. Leibowitz, S. F. Hypothalamic paraventricular nucleus interaction between α_2-noradrenergic system and circulating hormones and nutrients in relation to energy balance. Neurosci. Biobehav. Rev. 12:101–109; 1988.
20. Levine, A. S.; Morley, J. E. Neuropeptide Y: A potent inducer of consummatory behaviour in rats. Peptides 5:1025–1029; 1984.
21. Lundberg, J. M.; Terenius, L.; Hokfelt, T.; Martling, C. R.; Tatemoto, K.; Mutt, V.; Polak, J.; Bloom, S.; Goldstein, M. Neuropeptide Y (NPY)-like immunoreactive substance and catecholamines in some peripheral and central neurons. Acta Physiol. Scand. 116:477–480; 1982.
22. Maccarrone, C.; Jarrott, B. NPY: A putative neurotransmitter. Neurochem. Int. 8:13–22; 1986.
23. Moltz, J. H.; McDonald, J. K. Neuropeptide Y: Direct and indirect action on insulin secretion in the rat. Peptides 6:1155–1159; 1985.
24. Morley, J. E.; Hernandez, E. N.; Flood, J. F. Neuropeptide Y increases food intake in mice. Am. J. Physiol. 253:R516–R522; 1987.
25. Morley, J. E.; Levine, A. S. The pharmacology of eating behaviour. Annu. Rev. Pharmacol. Toxicol. 25:127–146; 1985.
26. Morley, J. E.; Levine, A. S.; Gosnell, B. A.; Kneip, J.; Grace, M. P. Effect of neuropeptide Y on ingestive behaviors in rat. Am. J. Physiol. 252:R599–R609; 1987.
27. Orosco, M.; Trouvin, J. H.; Jacquot, C.; Cohen, Y. The metabolites of aminergic neurotransmitters in mesodiencephalic regions in two models of obese animals. Biogenic Amines 2:59–63; 1985.
28. Sahu, A.; Kalra, P. S.; Kalra, S. P. Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides 9:83–86; 1988.
29. Sawchenko, P. E.; Swanson, L. W.; Grzanna, R.; Howe, P. R. C. Colocalization of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus in the hypothalamus. J. Comp. Neurol. 241:138–153; 1985.
30. Stanley, B. G.; Chin, A. S.; Leibowitz, S. F. Feeding and drinking elicited by central injection of neuropeptide Y. Evidence for a hypothalamic site of action. Brain Res. Bull. 14:521–524; 1985.
31. Stanley, B. G.; Daniel, D. R.; Chin, A. S.; Leibowitz, S. F. Paraventricular nucleus injections of peptide YY and NPY preferentially enhance carbohydrate ingestion. Peptides 6:1205–1211; 1985.
32. Stanley, B. G.; Kirkouli, S. E.; Lampert, S.; Leibowitz, S. F. Neuropeptide Y chronically injected into the hypothalamus: A powerful neurochemical inducer of hyperphagia and obesity. Peptides 7:1189–1192; 1986.
33. Stanley, B. G.; Leibowitz, S. F. Neuropeptide Y stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci. 35:2635–2642; 1984.
34. Tatemoto, K. M.; Carlquist, M.; Mutt, V. Neuropeptide Y. A novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 246:659–660; 1982.