EFFECT OF NEUROPEPTIDE Y ON BEHAVIOR AND ON THE TIME COURSE OF THE BRAIN NORADRENALIN LEVEL

E. V. Borisova, G. Szabo,
B. V. Zhuravlev, and G. Telegdi

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Much attention is now being paid to the study of the physiological role of neuropeptides in the regulation of mechanisms of goal-directed behavior in animals and man. Pancreatic polypeptides have been studied on quite a wide scale from this standpoint. It has been shown that most of them affect the mechanisms of realization of feeding behavior. Neuropeptide Y (NPY), isolated from frog brain and first described in 1982 [7], is one of the pancreatic polypeptides. This peptide, consisting of 36 amino acids, is widely distributed not only at the periphery, but also in the CNS [8]. Central injection of NPY, as several investigators have found, can induce hypotension, changes in electroencephalographic activity, and the circadian rhythm [9]; it may have a sedative action [2, 9], and determine consummatory, feeding, and drinking behavior [1, 5, 6]. Close interaction has been observed between NPY and catecholamine systems: a change in their concentration in brain structures following central injection of the peptide [3], and their coexistence together in nerve cells [4].

This paper describes an experimental analysis of the behavioral repertoire of rats following central injection of NPY, with a simultaneous study of its effect on the noradrenalin concentration in various structures of the rat brain.

EXPERIMENTAL METHOD

Experiments were carried out on 78 male CFY rats weighing 170-200 g. In the course of the work the rats were kept under standard lighting conditions and at a constant temperature and with free access to food and water. The experiments were carried out between 8 a.m. and 4 p.m., and the possibility of circadian changes in the endogenous level of the peptides was taken into account. Cannulas were inserted into the lateral cerebral ventricles of the rats 7 days before the experiment. Two concentrations of NPY ("Sigma") were used, namely 1 and 100 ng in 2 μl water. As the control, 0.9% NaCl was used.

There were two series of experiments. Series I included the study of behavioral effects of NPY when injected intraventricularly. Six groups of rats, with 6 animals in each group, received NPY in accordance with the scheme described below. The experiments of series II were devoted to measurement of the noradrenalin level in the rats' brain after injection of the peptide. These were 7 groups of rats in this series. Animals of groups 1, 2, and 3 received an injection of 1 ng, those of groups 4, 5, and 6 received 100 ng of the peptide. Rats of groups 1 and 3 were killed 15 min after injection by decapitation, those of groups 2 and 5, 30 min, and of groups 3 and 6, 60 min after the injection. Animals of group 7 (control) received NaCl. The brain was removed from the rats and, after appropriate morphological and biochemical treatment by a modified method of Schellenberg and Gordon, the adrenalin concentration was determined in the following structures: frontal cortex, hypothalamus, amygdala, hippocampus, and septum. The significance of the results were determined by Student's test and by nonparametric tests.

EXPERIMENTAL RESULTS

In the experiments of series I, the control NaCl solution was injected into the group of animals, and 100 ng of NPY was injected on the second day. Nothing was injected on the third day. After the injection the rats were placed

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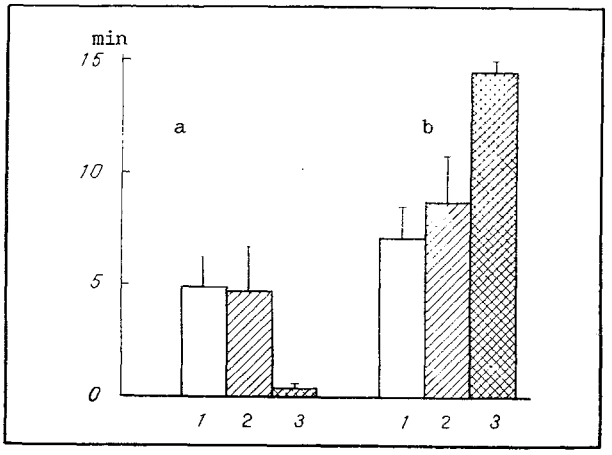

Fig. 1. Duration of OIB (a) and of rest periods (b) in intact animals (1) and after injection of control NaCL solution (2) and 100 ng of NPY (3).

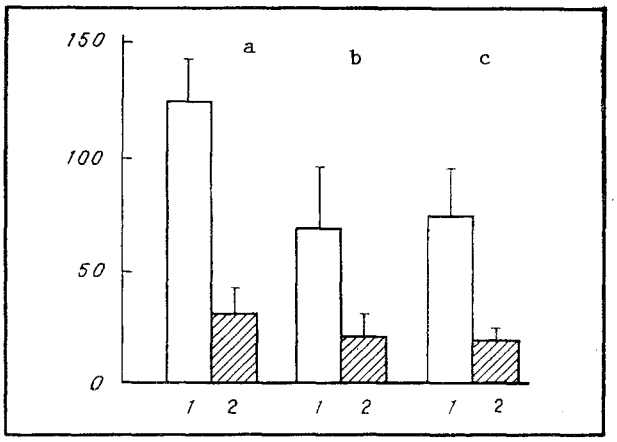

Fig. 2. Locomotor activity of three groups of rats before and after injection of 1 and 100 ng NPY, recorded during 30 min. Ordinate, number of trips around the chamber made by rats; a) control group, b) after injection of 1 ng, c) after injection of 100 ng NPY; 1) first 15 min after injection of substances, 2) second 15 min.

in the experimental chamber to which they had previously been accustomed, which contained feeding and drinking bowls, and which was placed in a darkened room. For 30 min the animals were tested for food and water consumption and various types of behavior were recorded, including total motor activity. The observations were aggregated 15 and 30 min after injection of the substances. As a results, very slight fluctuations in the duration of orienting-investigative behavior (during which the rats were in a state of rest sitting quietly or lying down) were observed in the intact animals and animals receiving the control NaCl solution. After injection of 100 ng of the peptide the duration of OIB was sharply reduced and the duration of the rest periods increased (Fig. 1). No changes were observed in feeding and drinking behavior after administration of the peptide.

The rats of group 2 were given an injection of NaCl solution, those of group 2 received 100 ng of NPY. These animals also were accustomed to the experimental chamber. In the rats receiving the peptide no changes were observed in feeding or drinking behavior. The duration of OIB was considerably reduced, especially in the first 15 min — from 3.5 ± 0.9 to 0.3 ± 0.1 min.

Rats of groups 4, 5, and 6 were tested by the open field method in the same chamber. These rats were placed in the chamber for the first time. One group was given an injection of NaCl solution, the second group received 1 ng and the third group 100 ng of the peptide. Just as in the previous groups, during the first and second 15 min the duration of OIB was sharply reduced (for the first 15 min: control 6.9 ± 1.4 min, 1 ng peptide 5.2 ± 1.5 min, 100 ng 5.1 ± 1.0 min; for the second 15 min: control 1.7 ± 0.1 min, 1 ng peptide 0.9 ± 0.1 min, 100 ng 1.2 ± 0.3 min), and the

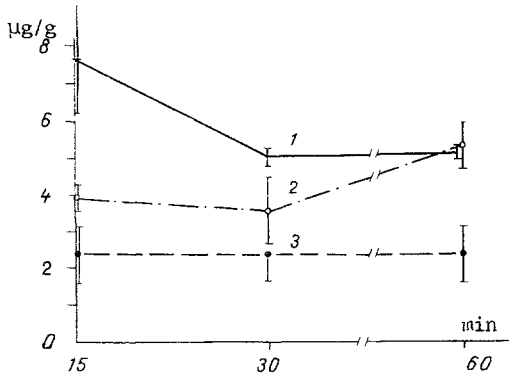

Fig. 3. Time course of noradrenalin level in hypothalamus of rats after injection of CPY. 1) injection of 1 ng of peptide; 2) injection of 100 ng; 3) control group.

TABLE 1. Noradrenalin Concentration in Amygdala and Hippocampus after Injection of 1 and 100 ng of NPY

Dose, ng	T	n	Noradrenalin, μg/g	σ
			Amygdala	
1	15	5	0,34±0,07	0,15
1	30	5	0,15±0,03	0,07
1	60	5	0,32±0,08	0,18
100	15	6	0,15±0,02	0,04
100	30	6	0,21±0,06	0,15
100	60	6	0,13±0,02	0,05
C	—	5	0,15±0,05	0,12
			Hippocampus	
1	15	5	0,22±0,03	0,07
1	30	6	0,24±0,03	0,07
1	60	6	0,18±0,03	0,07
100	15	6	0,15±0,03	0,07
100	30	6	0,23±0,04	0,09
100	60	6	0,26±0,06	0,15
C	—	4	0,47±0,10	0,21

Legend. T) time after injection of peptide, n) number of animals, σ) standard derivation, C) control group.

duration of the rest period was increased correspondingly. The locomotor activity of the animals was significantly reduced, especially in the first 15 min (Fig. 2). Changes in food consumption were not significant.

The experiments of series II showed no statistically significant deviations from the normal noradrenalin level in the frontal cortex and septum before or after injection of 1 and 100 ng of the peptide. Some fluctuations in the noradrenalin level were recorded in the region of the amygdala. In the hippocampus there was a tendency for the noradrenalin concentration to fall after injection of NPY (Table 1). Only in the hypothalamus was there any statistically significant increase in the noradrenalin concentration after injection of both 1 ng and 100 ng of the peptide; the increase was dose-dependent, moreover, in the first 30 min (Fig. 3).

It must be emphasized that the doses which we chose are much lower than those mentioned in the accessible literature [1, 6]. This may evidently be reflected in the behavioral effect obtained: inhibition of locomotor activity, weakening of OIB, lengthening of the period spent at rest, all of which correlated with changes in the noradrenalin concentration in the brain structures. In our view the doses chosen are more physiologically adequate, for we showed that low doses of NPY, administered centrally, inhibit catecholamine utilization, whereas high doses stimulate it [3]. According to some workers, an increase in catecholamine utilization gives rise to a whole series of changes in the secretion of certain hormones: the plasma TSH, STH, and prolactin levels are reduced, secretion of ACTH, aldosterone, and corticosterone is increased [3, 8]. Low doses, on the other hand, inhibit the utilization of some catecholamines and

do not give rise to any such changes [3]. On the basis of these results NPY can be regarded as an important neuroendocrine modulator, closely linked with the catecholaminergic systems of the body. It can accordingly be postulated that the behavioral effects recorded in response to central injection of low doses of NPY may be connected with activation of the catecholamine systems of the diencephalon, which is most marked in the hypothalamic region.

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HYPOTHALAMIC SEROTONIN CONCENTRATION IN ADULT MALE AND FEMALE AND NEONATALLY CASTRATED MALE RATS

N. A. Borisova, B. N. Manukhin, and G. P. Selivanova

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The concentration of serotonin and the rate of its synthesis in the brain of sexually mature male rats are significantly lower than in the corresponding female brain [8, 15, 16]. The principal target region for sex hormones in the brain is the hypothalamus — the regulating center of the gonadotrophic function of the pituitary gland [11]. Sex differences in serotonin concentration in the hypothalamus have been found by some workers [3, 5] during the first few days after birth. It has been suggested that sexual dimorphism in serotonin metabolism begins to appear during the "critical period" of sexual differentiation of the brain. However, the role of sex hormones in differentiation of the serotoninergic system of the brain has not been explained.

The aim of this investigation was to study the character of changes in the serotonin level in the anterior (cyclic center) and mediobasal (tonic center regulating gonadotrophic secretion) hypothalamus of adult rats after blocking the action of testicular hormones on it from the first day of postnatal life.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats aged 3-4 months: females in the diestrus stage (D1 and D2), intact and neonatally castrated males. The animals were killed at 3-4 p.m.

The concentration of serotonin (5-hydroxytryptamine; 5-HT) was studied separately in the anterior hypothalamus, including the preoptic region, and the mediobasal hypothalamus. 5-HT was determined by fluorometry of its condensation products with orthophthaleic aldehyde [2]. Fluorescence was measured on the MPF-4 spectrofluorometer (Hitachi, Japan) using excitation at 360 nm and emission at 457 nm.

Laboratory of Hormonal Regulation and Laboratory of Intercellular Interaction, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow.

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