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Ca^{++}-DEPENDENT SEROTONIN SECRETION FROM THE RAT HYPOTHALAMUS DURING ONTOGENY

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One of the most important functions of the serotoninergic system of the brain is its role in regulation of the secretion of pituitary trophic hormones. At the hypothalamic level serotonin (5-hydroxytryptamine; 5-HT) is involved as a neurotransmitter or neuromodulator in the regulation of secretion of adenohypophysiotropic neurohormones, whereas at the pituitary level, as a neurohormone it influences the secretion of pituitary trophic hormones [2].

In adult animals the main source of the 5-HT innervation of the hypothalamus is the dorsal and medial nuclei raphe in the midbrain. Differentiation of 5-HT-containing neurons in these nuclei takes place after the 13th day of embryonic life [11]. After the 16th day the fetal hypothalamus begins to be innervated by 5-HT fibers, and on the 18th day, serotonin-like neurons appear in it [10]. It is considered that in the early stages of ontogeny 5-HT plays the role of inducer of neurogenesis [5], but later it becomes involved in the regulation of function of the anterior lobe of the pituitary.

Data in the literature relate mainly to structural aspects of development of the serotonin system of the hypothalamus in ontogeny but give no idea about functional maturation of the serotoninergic fibers forming it.

The aim of this investigation was to study the formation of one of the most important properties of the hypothalamic serotoninergic fibers, namely Ca^{++}-dependent serotonin secretion in response to potassium depolarization, during ontogeny of the rat.

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EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats on the 16th, 17th, 18th, and 20th days of the prenatal period and also on rats aged 8-9 days and adult animals. Starting with the 20th day of fetal life only males were used in the experiments. To obtain a dated pregnancy, males were mated with females in the evening, and vaginal smears were taken in the morning. The day on which sperm was found in the smear was taken as the 1st day of pregnancy. Material was taken from the fetuses while the mother rat was anesthetized with pentobarbital, and from neonatal and adult animals after decapitation. The region of the brain, separated in the cold, included the mediobasal and anterior hypothalamus and also the septum and Broca's diagonal band, for these regions in adult animals are responsible for regulation of the gonadotrophic function [4]. The hypothalamus was divided in the mid-sagittal plane along the third ventricle into two equal parts, starting with the 17th day of prenatal life.

Fragments of the hypothalamus were placed in glass flasks with 2 ml of Krebs—Ringer bicarbonate buffer (pH 7.4) of the following composition (in mM): NaCl — 120; KCl — 4.8; $CaCl_2$ — 2; $MgSO_4$ — 1.2; KH_2PO_4 — 1.25; $NaHCO_3$ — 25; D-glucose — 10, ascorbic acid — 0.13, pargyline — 0.3.

After preincubation for 10 min at 37°C in an atmosphere of carbogen and with gentle shaking, the fragments were transferred into flasks containing ^{3}H-5-HT (specific activity 17-20 Ci/mmole, final concentration 25×10^{-9} M, "Amersham International") and incubated for 20 min. Uptake of radioactively labeled serotonin was stopped by cooling the incubation medium to 0°C. The fragments were washed several times with cold initial buffer and transferred into perfusion chambers, as described in [3]. Eight halves of hypothalami of 16-day fetuses, or four halves from 17-20-day fetuses, or two halves from neonatal or adult animals were placed into each chamber. The chamber was completely immersed in a water bath at 37°C. The perfusion solution was continuously aerated with carbogen. The volume of incubation medium in the chamber was 400 μl. A constant rate of flow of solution through the chamber (400 μl/min) was maintained by a peristaltic pump ("Zaling," Poland).

After a stabilizing period of 40 min, during which no eluate was taken for analysis, four 2-minute fractions were collected for determination of spontaneous 5-HT release. The medium was then replaced by fresh, containing a raised K^+ concentration (60 mM KCl), and four more fractions were collected (secretion induced in response to depolarization). K^+-depolarization continued for 8 min. After rinsing the tissue with the initial solution for 8-10 min, perfusion was carried out with a solution containing a high K^+ concentration but not containing Ca^{++} (in this case the medium also contained 0.5 mM EGTA), and four more fractions were collected. K^+-depolarization was repeated 2 or 3 times. Next, 400 μl from each fraction was transferred into flasks containing 10 ml of ZhS-8 scintillation fluid and radioactivity was measured by liquid scintillation spectrometry on an "Intertechnique-SL-30" counter (France). Induced secretion was calculated as the difference between radioactivity of the fractions before and after stimulation.

EXPERIMENTAL RESULTS

The use of an in vitro perfusion technique in this investigation enabled the time course of exogenous ^{3}H-5-HT secretion from fragments of the hypothalamus of fetal, neonatal, and adult rats to be traced. According to Milder et al. [6], rapid removal of secreted 5-HT with the flow of incubation medium virtually excludes its reuptake, so that there was no need to use uptake inhibitors. Specificity of uptake was determined by the fact that, in low concentration (10^{-9} M) ^{3}H-5-HT is taken up only by serotoninergic terminals [9]. In addition, in view of the rapid deamination of serotonin by monoamine oxidase [12], pargyline, an inhibitor of this enzyme, was added to the medium to prevent catabolism of the amine.

Preliminary experiments showed that spontaneous release of labeled 5-HT fell during the first 40 min of perfusion in all the experimental groups. The dynamics of induced serotonin secretion was therefore studied after a stabilization period of 40 min.

A study of the ability of the fetal rat hypothalamus to secrete assimilated ^{3}H-5-HT showed that it was spontaneously released after the earliest of the times chosen for study, namely the 16th day (Fig. 1), but no response to K^+-depolarization could yet be found. The response to depolarization, in the form of an increase in the rate of secretion of the exogenous mediator, first appeared on the 17th day of development. During repeated increases in the K^+ concentration a corresponding increase was observed in the rate of secretion of labeled serotonin into the medium. On removal of Ca^{++} from the medium, increased release of ^{3}H-5-HT did not take place in response to depolarization.

Ca^{++} is known to play a key role in the initiation of neurotransmitter secretion in adult animals. At the moment of depolarization Ca^{++} enters the terminal and also is mobilized from the intracellular storage depots, and triggers the secretion mechanism, resulting in increased activity of protein kinase, which phosphorylates proteins in the membrane of the vesicles and activates the system of microtubules and microfilaments [8]. It can be tentatively suggested that after

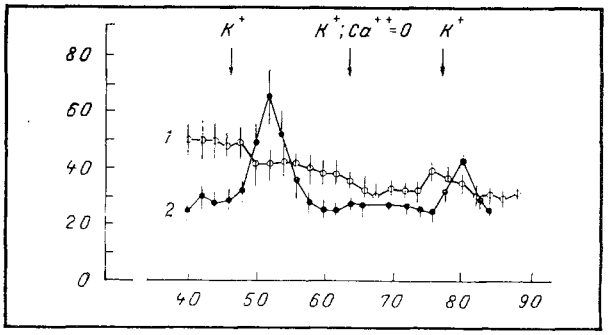

Fig. 1. Spontaneous and depolarization-induced secretion of ^{3}H-serotonin from fragments of fetal rat hypothalamus on the 16th and 17th days of development. K^+) Potassium depolarization in the presence of Ca^{++}, K^+; $Ca^{++} = 0$) potassium depolarization in the absence of Ca^{++}. 1) Fetuses on 16th day of development, 2) on 17th day. Abscissa, time (in min); ordinate, radioactivity (in cpm of 400 μl fraction/mg tissue of perfused fragment).

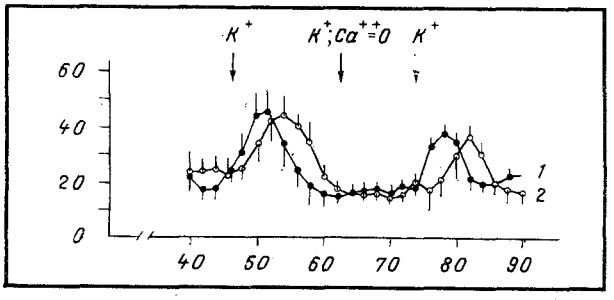

Fig. 2. Spontaneous and depolarization-induced secretion of ^{3}H-serotonin from hypothalamic fragments of fetal rats on 18th and 20th days of development. 1) Fetuses on 18th day, 2) on 20th day. Remainder of legend as to Fig. 1.

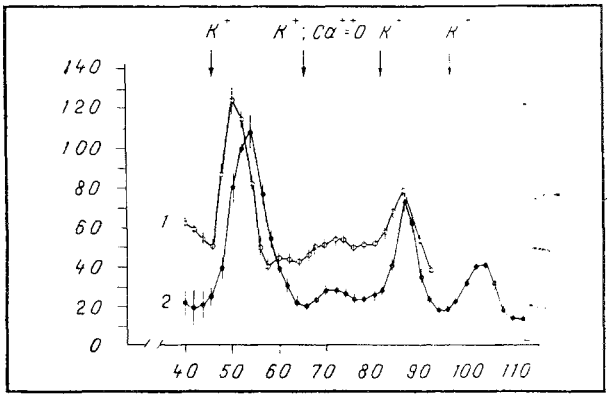

Fig. 3. Spontaneous and depolarization-induced secretion of ^{3}H-serotonin from hypothalamic fragments of neonatal and adult male rats. 1) Neonatal animals, 2) adult animals. Remainder of legend as to Fig. 1.

the 17th day of prenatal development the Ca^{++}-channels are already functioning and vesicular storage of serotonin is evidently taking place, because a Ca^{++}-dependent reaction to K^+-depolarization is observed.

The dynamics of ^{3}H-5-HT secretion from the fetal hypothalamus on the 18th and 20th days was similar to that on the 17th day (Fig. 2).

Release of ^{3}H-5-HT from the hypothalamus of neonatal and adult animals is shown graphically in Fig. 3. The rate of spontaneous secretion of the monoamine in neonatal animals was higher than in adults. The reaction to depolarization approached in magnitude the level observed in adult rats, and was distinctly Ca^{++}-dependent in character.

Our findings agree with the results of Nomura's investigation [7], which revealed high affinity of whole brain synaptosomes for 5-HT, and also depolarization-induced Ca^{++}-dependent secretion of labeled 5-HT, noradrenalin, and dopamine from whole brain sections from 18-day rat fetuses, and also with the findings of Barachovsky and Bradford [1], who studied 3-day cultures of whole brain of 17-day fetuses.

By the 17th day of prenatal development, controlled secretion of serotonin from hypothalamic nerve fibers thus becomes possible in response to depolarization. The response of neonatal animals to the depolarizing signal becomes the same as that of adult animals.

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TEMPERATURE STABILIZING EFFECT OF TOCOPHEROL ON RHODOPSIN IN THE PRESSURE OF FATTY ACIDS STUDIED BY DIFFERENTIAL SCANNING CALORIMETRY

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Hydrolysis products of phospholipids by phospholipase A_2, namely free fatty acids and lysophospholipids, together with lipid peroxidation (LPO) products, are factors modifying the properties of the lipid bilayer of membranes and also of a number of integral membrane proteins [6, 12]. The writers showed previously that free fatty acids (but not lysophospholipids), and also LPO products can lower the thermostability of the visual pigment rhodopsin in photoreceptor membranes [4, 10, 11]. It was also found that α-tocopherol (TP) has a protective action against thermal denaturation of rhodopsin, which is manifested during LPO only after preliminary introduction into the membranes, whereas in the case of free fatty acids, it is manifested on the addition of TP either before or after the fatty acid [4, 10].

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