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Brain as an endocrine source of circulating 5-hydroxytryptamine in ontogenesis in rats

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ABSTRACT

This study was aimed to test the authors' hypothesis stating that the developing brain before the closure of the blood brain barrier (BBB) operates as an endocrine organ that secretes classical neurotransmitters and neuropeptides into the general circulation. 5-Hydroxytryptamine (5-HT) was selected as a marker of brain endocrine activity though it is also secreted by peripheral organs. 5-HT was detected in blood of rats in a biologically active concentration at any studied age, from the 21st embryonic day till the 30th postnatal day. The brain was proven to be a source of circulating 5-HT before the BBB closure by showing that the 5-HT concentration in blood decreased significantly after the inhibition of 5-HT synthesis in the brain of neonates. The 5-HT concentration in blood was not diminished after the BBB closure, apparently due to compensatory increase of 5-HT secretion by peripheral sources. Thus, brain-derived 5-HT is delivered to the general circulation before the BBB closure being potentially capable of providing endocrine regulation of target organs.

1. Introduction

5-Hydroxytryptamine (5-HT) is a multipotent biologically active substance which is produced by the 5-HT neurons of the brain and enterochromaffin cells of the gastrointestinal tract, lungs and some other peripheral organs (Bertrand, 2006; Bonnin and Levitt, 2011; Gershon and Tack, 2007; Montange and Calas, 1988; Rindi et al., 2004; Steinbusch and Nieuwenhuys, 1983). Noteworthy that in adulthood the blood brain barrier (BBB) prohibits an exchange of monoamines including 5-HT, between the brain and the general circulation (Loizou, 1970; Sachs and Jonsson, 1975). 5-HT of the brain origin participates in the central regulation of neuroendocrine functions, circadian rhythmic activity, different types of behavior (motor behavior, memory, emotional behavior, learning) (Fuller and Clemens, 1981; Inouye and Shibata, 1994; Leander et al., 1998; Lowry et al., 2005; Rusak and Zucker, 1979; Weiner et al., 1988), while peripheral 5-HT contributes to the endocrine regulation of the cardio-vascular system and paracrine regulation of the gastrointestinal tract, respiratory system, etc. (Côté et al., 2004; De Clerck and Janssen, 1990; Eddahibi and Adnot, 2006; Frishman and Grewall, 2000; Gershon and Tack, 2007; Hayreh, 1999; MacLean et al., 2000; Yusuf et al., 2003).

In ontogenesis, the 5-HT neurons of the brain begin to synthesize and release 5-HT just after their origin, in rats from the 13th to the 15th embryonic day (E) (Ugrumov et al., 1986, 1989; Ugrumov, 1997; Wallace and Lauder, 1983). However, 5-HT begins to function as a neurotransmitter significantly later, after a final implementation of the interneuronal synaptic network, in rats by the end of the second postnatal week. During the intermediate so called "pre-neurotransmission" period of the brain development, 5-HT exerts autocrine and paracrine irreversible actions on differentiating neurons as a morphogenic or transcription factor (Azmitia et al., 1990; Lauder, 1993; Levin et al., 2006; Mirochnik et al., 2005; Nebigil et al., 2000; Sodhi and Sanders-Bush, 2004; Ugrumov, 1997; Whitaker-Azmitia, 1993).

Taking into account that the BBB is established at the latest stage of the brain development in ontogenesis, for monoamines in rats during the second postnatal week (Kostrzewa, 2007; Loizou, 1970; Miyaguchi et al., 1999; Sachs and Jonsson, 1975), we have hypothesized that before the BBB closure the brain neurons, including 5-HT neurons function as secretory cells and the brain operates as a multipotent endocrine organ, which provides an endocrine regulation of peripheral target organs and the brain itself (Ugrumov, 2010). If this hypothesis is correct, the developing brain before the BBB closure should be an endocrine source of dozens or even hundreds of neuropeptides and classical neurotransmitters, including 5-HT, serving as inductors of development.

Abbreviations: BBB, blood brain barrier; E, embryonic day; $HClO_4$, perchloric acid; HPLC-ED, high performance liquid chromatography with electrochemical detection; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; pCPA, p-chlorophenylalanine; P, postnatal day.

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It has been already shown that the developing brain before the BBB closure is a principal endocrine source of circulating dopamine and gonadotropin-releasing hormone (Ugrumov et al., 2005; Ugrumov et al., 2012). Indeed, the BBB closure in ontogenesis results in a drop of the concentration of these neurohormones in peripheral blood until almost undetectable level. In such a low concentration, neurohormones are not capable any more of exerting endocrine influence on the peripheral target organs and the brain.

The goal of this study was to continue testing our hypothesis about the developing brain as an endocrine organ before the BBB closure in ontogenesis. However, this time 5-HT is used as a marker of brain endocrine activity. In contrast to previous markers, dopamine and gonadotropin-releasing hormone, 5-HT should be secreted to the general circulation before the BBB closure not only from the brain but also from some peripheral organs. The objectives of this study were to estimate in rats: (i) the concentration and content of 5-HT in the blood, brain and the duodenum in ontogenesis; (ii) the 5-HT concentration in the blood, brain and the duodenum following the pharmacological inhibition of 5-HT synthesis in the brain in neonates before the BBB closure.

2. Materials and methods

2.1. Animals

Wistar rats, pregnant females as well as males and females on the 21st embryonic (E) day (day of conception being E1), on the third postnatal (P) day (day of parturition being P1), P4, P16 and P30 were used in this study. Sex of animals was determined by identification of gonads in fetuses and estimating of the anogenital distance in young rats. The animals were purchased from the Laboratory animal farm Stolbovaya RAMS (Moscow, Russia) and maintained at 21–23 °C in a 12 h light-dark cycle with free access to food and water.

2.2. Experimental procedures

2.2.1. Collecting of blood and tissues of intact animals

The blood was collected from the incised heart of intact animals under the pentobarbital anesthesia (40 mg/kg bw): (a) fetuses on E21, 58 males and 62 females; (b) rats on P4, 13 males and 11 females; (c) rats on P16, 14 males and 13 females; (d) rats on P30, 35 males and 35 females. For biochemical assay the blood was pooled from three fetuses on E21 as an individual sample, whereas using blood from each postnatal rat as an individual sample.

In addition to blood, the whole brain and the proximal third of the duodenum were dissected from intact fetuses at E21 and pups at P4, P16 and P30. Materials from each animal were used as individual samples. The tissue was weighted, frozen and maintained at −70 °C until assay with high performance liquid chromatography with electrochemical detection (HPLC-ED).

2.2.2. Animal treatments

In the first series of experiments p-chlorophenylalanine (pCPA) (Sigma, St. Louis, USA), an inhibitor of tryptophan hydroxylase and hence of 5-HT synthesis (Koe and Weissman, 1966), at the dose of 100 μg, 150 μg or 250 μg per animal in 10 μl of NaCl was intraperitoneally injected to 78 male rats on P3. Sixty three male rats, which received only saline intraperitoneally served as controls. The materials (blood, the whole brain and the proximal third of the duodenum) were collected from rats under anesthesia twenty four hours after the injection of pCPA in saline or only saline. The blood was fractioned for getting plasma and platelets separately. All collected tissues were weighted and kept at −70 °C until HPLC-ED assay.

In the second series of experiments 100 μg of pCPA in 2 μl of 0.9% NaCl was stereotactically injected to the lateral ventricles of 20 male rats on P3 (1.4 mm lateral to bregma, 2–2.5 mm deep into the nervous tissue) as it was described earlier (Ugrumov and Mitskevich, 1980). In the control 2 μl of 0.9% NaCl was injected to the lateral ventricles of the brain of 10 male rats of the same age. The blood, brain and the duodenum of control rats and those treated with pCPA, were collected under anesthesia 24 h after the injection. According to literature data, this period corresponds to a maximum decrease of tryptophan hydroxylase activity and 5-HT synthesis in the brain of adult rats (Gál et al., 1970; Koe and Weissman, 1966). The dissected brain and the duodenum were weighted and all the collected materials were frozen and kept at −70 °C until HPLC-ED assay.

In the third series of experiments 100 μg of pCPA in 2 μl of 0.9% NaCl was stereotactically injected to the lateral ventricles of 10 male rats on P3 as described above that was followed 23.5 h later by the intraperitoneal injection of 3-hydroxybenzyl hydrazine (Sigma, St. Louis, MO, USA), an inhibitor of aromatic L-amino acid decarboxylase (Carlsson and Lindqvist, 1973), at the dose of 100 mg/kg. Ten control animals of the same age have received intraventricularly 2 μl of 0.9% NaCl and 23.5 h later intraperiotoneally 3-hydroxybenzyl hydrazine at the dose of 100 mg/kg. The brain and duodenum of experimental and control rats were collected under anesthesia 24 h after the first injection.

All the experimental procedures were carried out in accordance with the regulations of the National Institute of Health in accordance with the Guide of Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and in accordance with the Directive of the European Communities Council of 24 November 1986 (86/609/EEC) for care and use of laboratory animals which were accepted by the Institute of Developmental Biology of the Russian Academy of Sciences.

2.2.3. Primary processing of collected materials

The blood in a volume of 300 μl was placed in a tube containing 30 μl of 5% ethylenediaminetetraacetic acid (Sigma, St. Louis, USA) and 10 μl of 10% sodium metabisulfite (Sigma, St. Louis, USA) and centrifuged at 80*g* for 10 min for getting plasma enriched with platelets. Then, the platelets were separated from plasma by repeated centrifugation at 700*g* for 10 min. Each plasma sample was added one-tenth volume of 1 N perchloric acid ($HClO_4$) to a final concentration of 0.1 N $HClO_4$, and 1 ng of alpha-methylserotonin, internal standard in 10 μl of 0.1 N $HClO_4$.

Each sample with platelets was added 80 μl of 0.1 N $HClO_4$, 10 μl (1 ng) of alpha-methylserotonin, and all samples were centrifuged for 20 min at 15200 g. The supernatant was collected, frozen and stored at −70 °C until assay of 5-HT and 5-hydroxytryptophan (5-HTP). Then, the number of platelets was counted in 10 μl of plasma enriched with these blood cells using the Goryaevs' chamber and the average content of 5-HT per 10^9 platelets was estimated as it was proposed earlier (Guicheney et al., 1985).

In order to determine the content of 5-HT and 5-HTP in the whole brain and the duodenum, dissected tissues were homogenized in 0.1 N $HClO_4$ using an ultrasonic homogenizer (L-666, MSE, England). Moreover, 10 μl of 0.2 N $HClO_4$, containing 1 ng of alpha-methylserotonin was added, followed by centrifugation at 15200*g* for 20 min. The supernatant was collected, frozen and stored at −70 °C up to HPLC-ED assay.

2.2.4. High performance liquid chromatography with electrochemical detection

HPLC-ED was used to measure 5-HT and 5-HTP. Frozen tissues were warmed, centrifuged at 80*g* for 10 min at 4 °C and the