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Journal of Thermal Biology 32 (2007) 188–192

Journal of THERMAL BIOLOGY

www.elsevier.com/locate/jtherbio

Expression of the 1A and 2A serotonin receptor genes in the brain of rats adapted to warm and cold

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Received 13 September 2006; accepted 3 January 2007

Abstract

1. The effect of long-term thermal adaptation on expression of 5-HT_{1A} and 5-HT_{2A} receptor genes was studied by quantitative RT-PCR in brain regions of rats adapted for 5 weeks to warm (+20 to +22 °C) or cold (+4 to +6 °C).
2. No differences were found in the 5-HT_{1A} receptor mRNA levels in the rats of two groups.
3. The 5-HT_{2A} receptor mRNA levels in cold-adapted rats in comparison to warm-adapted rats increased in hypothalamus and decreased in frontal cortex, but were unaltered in hippocampus.

Keywords: Long-term thermal adaptation; 5-HT_{1A}; 5-HT_{2A} receptors; mRNA; Rat

1. Introduction

Temperature adaptation results in changes in the function of many organs and systems (Hart, 1964; Jansky, 1978; Bruck and Zeisberger, 1987; Banet, 1988; LeBlanc, 1992). Together with changes in the organs responsible for heat production and heat loss, the thermosensitivity of organism also differs at both the peripheral and central structural levels (Kozyreva, 2006). Brain serotonin may play an important role in the responses of homeotherms to temperature change, but data on the role of serotonin in the maintenance of thermal homeostasis remain controversial. There is abundant evidence indicating that temperature affects the brain serotonin level and its turnover. These effects depend on an orientation (cold or heat), intensity (cold as +10 or 0 °C) and duration (30 min or 5 h) of temperature influence (Welch, 1968; Harri and Tirri, 1969; Williams and Moberg, 1975; Myers, 1981; Roth, 1989, cited by Zeisberger, 1990; Harikai et al., 2003; Ishiwata et al., 2004). However, it is difficult to find any noticeable regularity in the temperature and serotonin relationship. Furthermore, alterations of thermoregulatory parameters have been reported after the experimental modification of brain serotonin levels and these parameters varied depending on an experimental procedure and animal species used (Feldberg and Myers, 1964; Bligh et al., 1971; Ishiwata et al., 2004). These different outcomes are probably because the regulatory functions of serotonin act via the specific serotonin receptors and the state of these receptors may change.

At present, 14 types and subtypes of serotonin receptors have been distinguished. Every receptor subtype is characterized by own amino acid sequence, enchromosomal localization of the encoding gene, mechanism of signal transduction and functional features (Saudou and Hen, 1994; Barnes and Sharp, 1999). The 5-HT_{1A} and 5-HT_{2A} serotonin receptors are the most studied. These receptors are involved in the regulation of numerous physiological responses (Barnes and Sharp, 1999; Popova and Kulikov, 2003) and it is precisely with their activation that most researchers identify the induced hypo- and hyperthermia in

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0306-4565/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtherbio.2007.01.001

animals (Goodwin and Green, 1985; Gudelsky et al., 1986; Aulakh et al., 1988; Mazzola-Pomietto et al., 1997; Knapp et al., 1998).

There are data indicating that thermal conditions modify the response of the organism to serotonin agonists and antagonists (Bligh et al., 1971; Popova and Konusova, 1985; Oerther, 2000; Nicholas and Seiden, 2003). It follows that at different environmental temperatures, either the state of the serotonin receptors changes or the sensitivity and (or) function of the thermoregulatory neurons (Nicholas and Seiden, 2003) that express these receptors become modified. And this modification may arise under the effect of the serotonin receptors.

Indeed, the hypothalamic thermoregulatory center receives serotonergic innervation (Dahlstrom and Fuxe, 1964) and most of the thermosensitive hypothalamic neurons can respond to microinjected serotonin (Hori and Nakayama, 1973; Watanabe et al., 1986), i.e. they contain postsynaptic serotonin receptors. Thus, it appears reasonable to assume that the changes in thermosensitivity of the hypothalamic neurons after long-term thermal adaptation may also be mediated via the state of serotonin receptors.

Proceeding on these assumptions, the aim of the present study was to analyze the expression of the 5-HT_{1A} and 5-HT_{2A} receptor genes in some brain regions in animals adapted to different temperatures.

2. Methods

Male Wistar rats ($n = 20$) weighting 200–250 g, obtained from the Institute of Cytology and Genetics (Siberian Branch of Russian Academy of Sciences, Novosibirsk) were used throughout. Thermal adaptation was performed according to Hart's protocol (Hart, 1964): the animals were housed for 5 weeks in single cages $60 \times 40 \times 20$ cm under a 12:12 h light–dark cycle (light on at 8.00 AM) with free access to food and water in temperature regulated rooms. The temperatures of the rooms were maintained at +4 to +6 °C for cold and at +20 to +22 °C for warm adaptations. All animals received the special fodder for laboratory rats "PK-120" produced by "Laboratorsnab" (Moscow, Russia). All experimental procedures were in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

After 5 weeks of adaptation, the cold and warm adapted rats were decapitated, their brains were rapidly removed on ice and the frontal cortex, the hypothalamus and the hippocampus were isolated. The choice of the hypothalamus was based on its crucial role in the central regulation of body temperature in homeotherms. The frontal cortex and hippocampus were selected because of the highest density and expression of the 5-HT_{2A} (cortex) and 5-HT_{1A} (hippocampus) receptors (Barnes and Sharp, 1999).

The samples of frontal cortex were received by vertical section at the distance of 1.2–1.5 mm from *polus frontalis* (previously the olfactory bulbs with frontal parts of olfactory tracts were removed). The weight of samples from two cerebral hemispheres was ≈40 mg. Then the optic chiasma and the hypophysis were removed. The hypothalamic area including the *tuber cinereum* with its preoptic part and the mamillary bodies was isolated by the section of depth ≈1.5 mm. The weight of the sample was ≈30 mg. To take the hippocampus, the *corpus callosum* was cut along the *fissura longitudinalis cerebri* and cerebral hemispheres were moved apart, cortex was partially moved aside to uncover the hippocampus. The weight of two hippocampus was 100–120 mg. Brain tissue samples were placed in sterile eppendorf tubes, frozen under liquid nitrogen and stored at −66 °C until RNA extraction.

The expression of the 5-HT_{1A} and 5-HT_{2A} receptor genes was assayed by quantitative RT-PCR, as described in detail previously (Kulikov et al., 2005). Total RNA was isolated by guanidinium thiocyanate–phenol–chloroform extraction (Chomczynski and Sacchi, 1987), its concentration was determined by absorbance at 260 nm and its purity was estimated by 260/280 ratios. The RNA integrity was evaluated by electrophoresis in 0,8% agarose gel. Only RNA samples with clearly visible 28S and 18S rRNA bands were used. The absence of DNA contamination in RNA preparations was checked by PCR analysis using primers for β-actin and 1 μl of total RNA diluted five times. No sign of genomic β-actin fragment was detected by 34 cycles of amplification.

Total RNA, 8 μl (1 μg), was mixed with 180 ng of random hexamer primer and 2.25 μmol of sterile KCl in the volume of 16 μl, denatured at 94 °C for 5 min, annealed at 41 °C for 15 min, and then 15 μl of mixture containing reverse transcriptase M-MLV (200 U) (Biosan, Russia), Tris HCl (pH 8.3, 0.225 μmol), dNTP mixture (0.015 μmol of each) (Sibenzyme, Russia), dithiothreitol (0.225 μmol) and $MnCl_2$ (0.03 μmol) were added. The mixture (at the final volume of 31 μl) was incubated at 41 °C for 60 min. The synthesized cDNA was stored at −20 °C.

An aliquot of 2 μl of cDNA was mixed with 2 μl of PCRx10 buffer, 1 μl of dNTP mixture, 0.03 μmol of $MgCl_2$, 5 μl of mixture of appropriate sense and antisense primers (for 5-HT_{1A}, 5-HT_{2A} or β-actin corresponding to 250 pmol of each), 1 U of *Taq* polymerase (Sibenzyme, Russia) and sterile water to the final volume of 20 μl. PCR was carried out in Hybaid Omn-E (UK) thermocycler in the following conditions: (i) 5 min at 94 °C; (ii) 40 s at 94 °C, 40 s at appropriate anneal temperature (Table 1), 40 s at 72 °C, 32 (receptors in cortex and hippocampus), 34 (receptors in hypothalamus) or 24 (β-actin) cycles; (iii) 4 min at 72 °C.

The control tubes containing 2 μl of external exogenous standard DNA (100 ng/μl for β-actin or 10 ng/μl for the receptor) instead of cDNA and the same reagent mixture were amplified together with the corresponding sample tubes. We used rat genomic DNA as the external standard containing 200 copies of the target genes per 1 ng. Negative controls included the same reagent mixture except that cDNA or DNA was omitted.