Biochimie 105 (2014) 211–215

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Short communication

Serotonin activates cell survival and apoptotic death responses in cultured epithelial thyroid cells

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ARTICLE INFO

Article history:
Received 21 May 2014
Accepted 25 June 2014
Available online 2 July 2014

Keywords:
Rat thyroid epithelial cell line
Serotonin
5-HT2A receptor
SERT
ERK1/2

ABSTRACT

Anatomic and physiological interactions between central serotonergic system and thyroid gland are well established. However, the effects of locally available serotonin on the thyroid functions are poorly known. Here, we first demonstrate the expression of serotonin transporter SERT and 5-HT2A receptor subtype in rat thyroid epithelial cell line FRT both at mRNA and protein levels. In order to investigate the molecular mechanisms of serotonin action, FRT cells were exposed to increasing concentrations of the amine. Low concentrations of serotonin (up to 5 μM) enhanced FRT cell growth, and ERK1/2 and SMAD2/3 phosphorylation. Cell exposure to the selective 5-HT2A receptor agonist DOI recapitulated the effects of 5-HT on ERK1/2 phosphorylation. By contrast, administration of M100907, a specific 5-HT2A receptor inhibitor, prevented 5-HT induced ERK1/2 activation. On the other hand, high doses of serotonin (50 μM up to 1 mM) activated a caspase-3 mediated apoptosis of cells. Overall, our findings demonstrate that low levels of serotonin, interacting with 5-HT2A receptor, are able to activate proliferative signals in the thyroid epithelial cells, while high levels of serotonin cause pro-apoptotic responses, thus suggesting an active role of the amine in the thyroid functions and disorders.

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) is an ubiquitous monoamine which plays important roles in the development of neural and non-neural tissues of both vertebrate and invertebrate species [1-2]. The amine acts through 14 main receptor proteins which are divided into seven classes (5-HT1R to 5-HT7R) on the basis of their structural and operational properties. Both morphogenetic and neurotransmitter activities of 5-HT are strictly regulated by its transporter SERT which modulates the amine concentration in the extracellular fluid, thereby limiting its action on receptors.

In recent years, a growing interest on the role of 5-HT in thyroid function and disease is emerging. The interaction of the thyroid and serotonergic system has been suggested as a potential underlying mechanism of action for many developmental processes and psychiatric pathologies [3-4]. Reduced platelet content of 5-HT has been found in hypothyroid patients [5]. Loss of cortical 5-HT2AR appears to be the main consequence of hypothyroidism [6]. Protracted expression of the transporter SERT and altered thalamocortical projections in the barrel field of hypothyroid rats were observed [7]. Neonatal treatment with drugs affecting the maturation of serotonergic neurons in rats is responsible for an impaired thyroid function [8]. However, while a correlation between the central serotonergic system and thyroid disorders has been established, the role of locally available 5-HT on thyroid functionality is still an open issue.

In order to get more insights into 5-HT action on the gland, in this study, we evaluated 5-HT effect on the growth and survival of Fischer Rat Thyroid (FRT) epithelial cell line. We first tested whether FRT cells express SERT and 5-HT2AR. The presence of mRNAs coding for SERT and 5-HT2AR in FRT cells was investigated by RT-PCR analysis, using the following primers: forward 5′-C CTGCAGATCCATCAGTCTA-3′ and reverse 5′-TATTGGAAAAGCCGT AGCTG-3’ for SERT; forward 5′-TTTGAGAGGGGCTCTCTGGT-3′ and reverse 5′-TACACAGTGTTAATGCCATC-3′ for 5-HT2AR. As internal control, amplification of β-actin mRNA was carried out in parallel in each sample, using the primer pair: forward 5′-GGCACCCAGCAC AATGAAGAT-3′ and reverse 5′ CCTTCACCGTTCCAGTTTTTA-3′. As a

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http://dx.doi.org/10.1016/j.biochi.2014.06.020
0300-9084/

negative control for all reactions, distilled water was used in place of cDNA. The PCR products were separated on a 2% agarose gel and visualized by ethidium bromide, using a 1 kb DNA ladder to estimate the band sizes. This analysis resulted in the amplification of specific DNA fragments of 995 bp for SERT (Fig. 1A, left top) and of 531 bp for 5-HT2AR (Fig. 1A, right top), respectively, both in FRT cell samples (lanes 1) and in rat brain samples (lanes 2) used as a positive control. No amplification products were obtained when distilled water was used in place of the cDNA (negative control, lanes 3). A 357 bp amplification product was obtained from the β-actin cDNA in all tested samples (Fig. 1A, bottom).

The presence of the two proteins in the FRT cells was assessed by Western blotting. The use of a specific rabbit polyclonal antibody (AB1594P, Chemicon International Inc.) raised against a peptide mapping a 15 amino acid sequence between trans-membrane domains 7 and 8 of rat SERT allowed to detect in the cellular extracts a major band of approximately 94 kDa (Fig. 1B, left, lane 1). Rat SERT is a dodeca trans-membrane protein of 630 amino acids showing two canonical sites for N-linked glycosylation in the extracellular portion [9]. The 94 kDa electrophoretic band probably corresponds to a highly glycosylated SERT isoform. This finding is consistent with previous results on SERT expression in mammalian tissues and cultured cells [10,11]. Furthermore, the use of a rabbit polyclonal antibody (sc-50397, Santa Cruz Biotechnology) directed toward the amino acid sequence 1-75 mapping the N-terminal extracellular domain of the murine 5-HT2AR demonstrated the presence in the cellular extracts of the protein with the expected molecular weight of 55 kDa (Fig. 1B, right, lane 1). The expression of both SERT and 5-

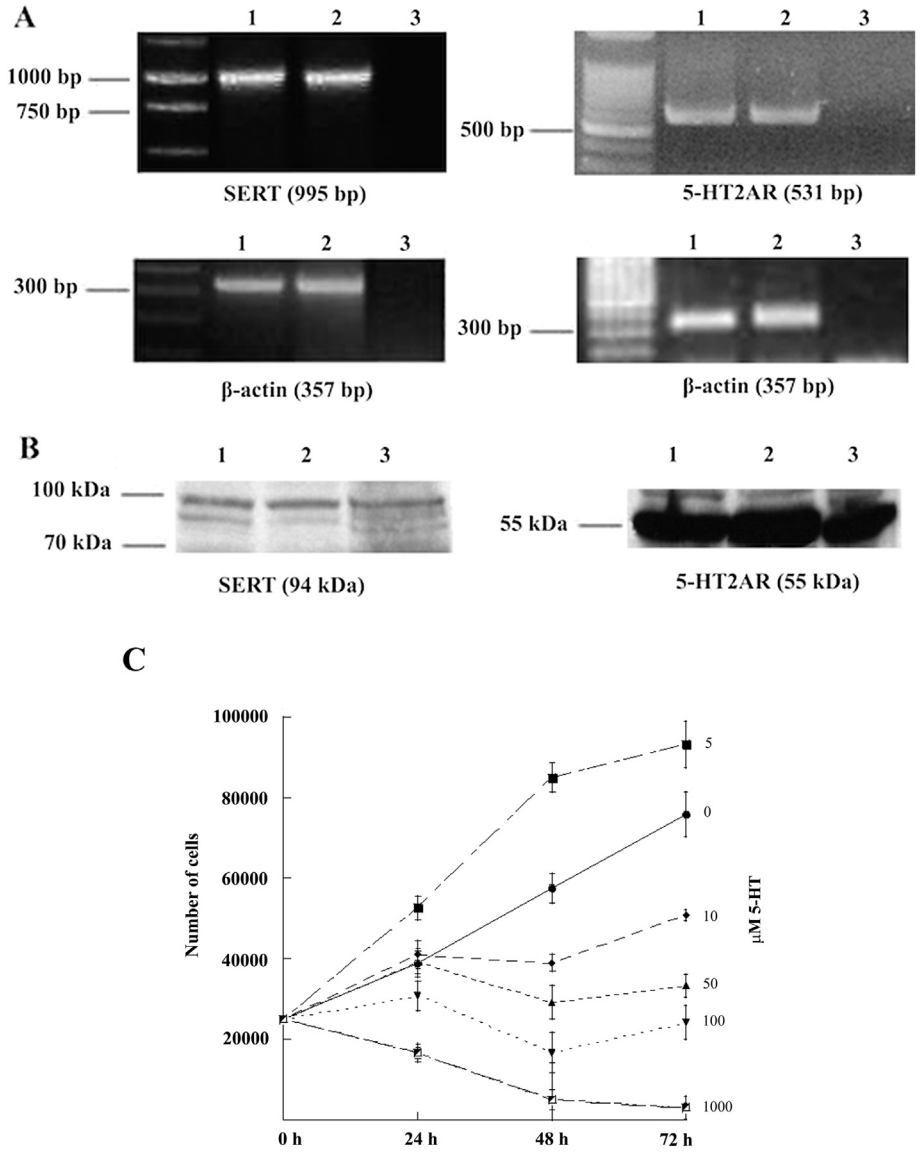

Fig. 1. A and B. Expression of SERT and 5-HT2AR by FRT cells. A. RT-PCR for SERT (left top) and 5-HT2AR (right top): lanes 1, FRT cells; lanes 2, rat brain homogenates; lanes 3, negative control (no cDNA input). Bottom left and right: β-actin mRNA transcripts (internal control). B. Western blotting for SERT (left) and 5-HT2AR (right): lanes 1, FRT cell lysates; lanes 2, mouse heart homogenates; lanes 3, rat brain samples. Similar results as those shown in panels A and B were obtained from four separate experiments of identical design. C. Effect of 5-HT on FRT cell growth. Cells were seeded in 24-well plates, grown overnight in complete medium, washed, and grown for 24 h in medium containing 1% FCS. After incubation, cells were treated with increasing doses of 5-HT. Control cells were treated with the medium alone. After 24, 48 and 72 h of incubation at 37 °C, the cells were trypsinized, and the number of alive cells, re-suspended in a solution of trypan blue, was determined by direct count using a hemocytometer. The data reported in panel C are the means of three independent experiments performed with each sample in replicate of three. Error bars indicate standard errors of the means.