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Hyperthyroidism changes nociceptive response and ecto-nucleotidase activities in synaptosomes from spinal cord of rats in different phases of development

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Abstract

Changes in transport, receptors and production of extracellular adenosine have been observed after induction of hyperthyroidism. Adenosine is associated with inhibitory actions such as reduction in release of excitatory neurotransmitters and antinociception at spinal site. In contrast, ATP acts as an excitatory neurotransmitter and produces pronociceptive actions. ATP may be completely hydrolyzed to adenosine by an enzyme chain constituted by an ATP diphosphohydrolase and an ecto-5′-nucleotidase, as previously described in the spinal cord. Thus, we now investigated the effects of the hyperthyroidism on adenine nucleotide hydrolysis in the spinal cord and verified the nociceptive response in this pathology during different phases of development. Hyperthyroidism was induced in male Wistar rats, aged 5, 60 and 330 days by daily intraperitoneal injections of L-thyroxine (T4) for 14 days. Nociception was assessed with a tail-flick apparatus. Rats starting the treatment aged 5 days demonstrated a significant increase in ADP and AMP hydrolysis and increased tail-flick latency (TFL). In contrast, in the spinal cord from hyperthyroid rats aged 60 and 330 days old, the hydrolysis of ATP, ADP and AMP were significantly decreased. Accordingly, the tail-flick latency was decreased, indicating a hyperalgesic response. These results suggest the involvement of ecto-nucleotidases in the control of the hyperthyroidism-induced nociceptive response in rats at distinct developmental stages.

Keywords: Hyperthyroidism; Adenosine; Ecto-nucleotidases; L-thyroxine; Development; Nociception

1. Introduction

Adenine nucleotides are known to be released in the extracellular space, where they act as important molecules for signaling (Vizi and Sperlagh, 1999). Extracellular ATP induces various biological responses, such as neurotransmission in both the peripheral and central nervous systems (Cunha and Ribeiro, 2000), excitability of spinal cord neurons (Sun et al., 1992), cell death via activation of P2Z/$P2X_7$ receptors (Schulze-Lohoff et al., 1998; Harada et al., 2000) and activation of pain pathways in the spinal cord (Tsuda et al., 1999). It has been demonstrated that extracellular ATP is hydrolyzed by an ATP diphosphohydrolase (apyrase, NTPDase, EC 3.6.1.5) in synaptosomes of the peripheral and central nervous systems (Battasttini et al., 1991; Sarkis and Saltó, 1991) generating AMP, which is

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later hydrolyzed by an ecto-5′-nucleotidase (EC 3.1.3.5) producing adenosine in the synaptic cleft. Alterations in ATP diphosphohydrolase and 5′-nucleotidase activity appear to be related to diverse situations such epilepsy in brain synaptosomes and chronic stress in the spinal cord (Bonan et al., 2000; Torres et al., 2002). These enzymes control the availability of extracellular nucleotides, the interaction of nucleotides at their respective receptors and, consequently, the biological effects mediated by this interaction (Chen and Guidotti, 2001).

Adenosine acts as a neuromodulator primarily though inhibition of excitatory neurotransmitter release (Dunwiddie and Masino, 2001). The inhibitory actions of adenosine are mainly mediated by activation of A_1 adenosine receptors (Fredholm and Dunwiddie, 1988), which are present in spinal cord (Choca et al., 1987). It has been proposed that adenosine is involved in physiological pain control at the spinal cord level and contributes to the action of opioid antinociception (Sollevi, 1997; Sweeney et al., 1989). In addition, adenosine may play a role in the pathophysiology of neuropathic pain, where pain-signaling mechanisms are altered (Guieu et al., 1996). The responses of adenosine receptors in relation to pain control are mediated by changes in cyclic AMP levels, inhibition of presynaptic voltage-gated calcium channel and via the activation of postsynaptic potassium channels (Salter et al., 1993; Sawynok, 1998). These results suggest a role of adenosine receptors in the modulation of both acute and chronic pain.

Furthermore, the extracellular levels of adenosine can be altered by a diverse array of pathological and physiological stimuli (Dunwiddie and Masino, 2001). Previous studies demonstrated that the thyroid hormones may modulate both nucleoside transporters and adenosine receptors of the A_1 subtype in the central nervous system (Fideu et al., 1994). In addition to their well-established role in cellular metabolism, thyroid hormones have critical effects upon cellular differentiation, growth, sensibility and synthesis of neurotransmitters (Engstron et al., 1974) and in vitro modulation of ecto-nucleotidase activities in brain synaptosomes (Matos et al., 2002). Disorders involving thyroid hormones are common and can be accompanied by severe symptoms. Manifestations of hyperthyroidism include anxiety, nervousness and tremulousness, irritability, tachycardia, emotional lability, physical hyperactivity, weight loss, increased perspiration, insomnia, weak muscles, increase in metabolic routs and, in serious situations, seizures (Orgiazzi and Mornex, 1990; Sarkar and Ray, 1994). Furthermore, hyperthyroidism is associated with an increase in the transport and metabolism of adenosine and a simultaneous decrease in membrane ecto-5′-nucleotidase activity in heart, altering this important endogenous cardioprotective mechanism (Smolenski et al., 1995).

Since thyroid hormones influence different biological systems, including the adenosine transport, and both adenosine and ATP are involved in the modulation of pain pathways in the spinal cord, the present study is aimed at investigating the effects of hyperthyroidism on the pain threshold and correlate the nociceptive mechanisms with the adenine nucleotide hydrolysis in spinal cord during different phases of development.

2. Materials and methods

2.1. Induction of hyperthyroidism

Male Wistar rats (*Rattus norvegicus*), weighing 6–7 g (5-day-old rats), 220–300 g (60-day-old rats) and 420–580 g (330-day-old rats) were used throughout this study. Animals were housed in cages with food and water available ad libitum and were maintained under a 12-h light/12-h dark cycle (light on at 07:00 a.m.) at a room temperature of 25 °C. Hyperthyroidism was induced by daily intraperitoneal injections of L-thyroxine (T4), 25 μg/100 g body mass, for 14 days (Friberg et al., 1985). T4 was dissolved using 0.04 M NaOH, and the final solution was prepared with 0.9% saline solution. Control animals received intraperitoneal injections of 0.9% saline solution. Animals were killed by decapitation 24 h after the last injection. Procedures for the care and use of animals were adopted according to the regulations published by the Brazilian Society for Neuroscience and Behavior (SBNeC).

2.2. Subcellular fractionation

The rats were killed by decapitation, and the spinal cord was rapidly removed and gently homogenized in 10 volumes of an ice-cold medium consisting of 320 mM sucrose, 0.1 mM EDTA and 5.0 mM HEPES, pH 7.5 with a motor-driven Teflon glass homogenizer. The synaptosomes were isolated as described previously (Nagy and Delgado-Escueta, 1984). Briefly, 0.5 mL of the crude mitochondrial fraction were mixed with 4.0 mL of an 8.5%. Percoll solution was layered onto an isosmotic Percoll/sucrose discontinuous gradient (10%/20%). The Percoll interface was collected with a wide-tip disposable plastic transfer pipette, and the synaptosome pellet was resuspended to a final protein concentration of approximately 0.5 mg/mL. The material was prepared fresh daily and maintained at 0–4 °C throughout preparation.

2.3. Enzyme assays

The reaction medium used to evaluate ATP and ADP hydrolysis in the synaptosomal preparation was essentially as described previously (Battasttini et al., 1991) and contained 5.0 mM KCl, 1.5 mM $CaCl_2$, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose and 45 mM Tris–HCl buffer, pH 8.0, in a final volume of 200 μL.

The reaction medium used to assay ecto-5′-nucleotidase activity contained 10 mM $MgCl_2$, 100 mM Tris–HCl, pH