Hyperthyroidism increases adenosine transport and metabolism in the rat heart

Ryszard T. Smolenski*, Magdi H. Yacoub and Anne-Marie L. Seymour
Department of Cardiothoracic Surgery, National Heart and Lung Institute at Harefield Hospital, Harefield, Middlesex UB9 6JH UK

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Abstract

Hyperthyroidism induces a number of metabolic and physiological changes in the heart including hypertrophy, increase in inotropic status, and alterations of myocardial energy metabolism. The effects of hyperthyroidism on adenosine metabolism which is intimately involved in the control of many aspects of myocardial energetics, have not been clarified. The aim of this study was thus to evaluate the potential role of adenosine in the altered physiology of the hyperthyroid heart. Transport of adenosine was studied in cardiomyocytes isolated from hyperthyroid and euthyroid rats. Activities of different enzymes of purine metabolism were studied in heart homogenates and concentrations of nucleotide and creatine metabolites were determined in hearts freeze-clamped in situ.

Both transport of adenosine into cardiomyocytes and the rate of intracellular phosphorylation were higher in the hyperthyroid rat. At 10 µM concentration, adenosine transport rates were 275 and 197 pmol/min/mg protein in hyperthyroid and euthyroid cardiomyocytes respectively whilst rates of adenosine phosphorylation were 250 and 180 pmol/min/mg prot. An even more pronounced difference was observed if values were expressed per number of cells due to cardiomyocyte enlargement. Hyperthyroidism was associated with a 20% increase in adenosine kinase, 30% decrease in membrane 5'-nucleotidase and 15% decrease in adenosine deaminase activities measured in heart homogenates. In addition there was a substantial depletion in the total creatine pool from 63.7 to 41.6 µmol/g dry wt, a small decrease in the adenylate pool (from 27.2 to 24.3 µmol/g dry wt) and an elevation of the guanylate pool (from 1.22 to 1.36).

These results show that adenosine transport and phosphorylation capacity is enhanced in hyperthyroidism. These may represent a feedback response to accelerated nucleotide degradation suggested in turn by the decrease in steady-state adenine nucleotide content. The decrease in membrane 5'-nucleotidase activity may represent another feature of hypertrophy where the cell surface to cell volume ratio decreases. The decrease of this activity may modify the conversion of extracellular nucleotides to adenosine and consequently reduce endogenous cardioprotection. (Mol Cell Biochem 143: 143–149, 1995)

Key words: adenosine, adenosine transport, hyperthyroidism, cardiomyocytes, membrane-5'-nucleotidase, adenine nucleotides, creatine compounds

Introduction

Thyroid hormone controls a number of metabolic and physiological functions in cardiac and other tissues. Hyperthyroidism causes an increase in heart rate, contractility and cardiac output, raising the inotropic status of the heart and is accompanied by enlargement of the myocytes [1–4]. This hypertrophy distinguishes the heart from a normal one. The adaptations that occur lead initially to a greater efficiency in the heart. The rate of protein synthesis is enhanced as is the oxygen consumption [2, 5, 6]. In addition, there is evidence for alterations in energy metabolism with a reduction in myocardial energy reserve, an enhanced capacity for glycolysis and an increased expression of the Na+-K+ ATPase [3, 7–9].
Adenosine is directly involved in the regulation of myocardial energetics. It can serve as a precursor of ATP, regulate the supply of blood to the heart and counteract excessive catecholamine stimulation [10-12]. As previous studies have identified substantial modifications in myocardial energy metabolism in the hyperthyroid heart, there may also be changes in adenosine metabolism. Myocyte enlargement may result in a relative increase in adenosine transport sites in the membrane as has been shown with glucose transport [10]. Alternatively, there may be no change in expression of transport protein leading to a down regulation of adenosine transport similar to that seen with β-receptors [13]. Changes in enzymatic patterns may result in modification of the potential for adenosine production and in turn may disturb the physiological mechanisms mediated by adenosine. In particular, the antagonistic effect of adenosine on catecholamine stimulation may be of importance in relation to the altered inotropic status of the hyperthyroid heart. It is possible that a decrease in adenosine production may be one mechanism underlying the long recognised increased sensitivity of the hyperthyroid heart to catecholamine stimulation [13].

The aim of this study was to determine changes in adenosine transport capacity and metabolism in hyperthyroid hearts to evaluate the potential role of adenosine in the altered physiology of the heart in hyperthyroidism. These results indicate that adenosine transport and phosphorylation capacities are enhanced, which may balance the accelerated turnover of adenine nucleotides resulting from the increased metabolic rate of the hyperthyroid heart. In addition, the reduced capacity to generate extracellular adenosine may alter endogenous cardioprotective mechanisms in the heart.

**Materials and methods**

**Induction of hyperthyroidism**

Male Wistar rats, weighing 250-350 g were used in this study. Hyperthyroidism was induced as described previously [3], by daily intraperitoneal injections of L-thyroxine (T₄), 35 μg/100 g body weight, for 7 days. Control animals received vehicle (0.01 M NaOH) only.

**Cardiomyocyte adenosine transport and metabolism**

Cardiomyocytes were isolated from hearts of hyperthyroid and euthyroid rats using a collagenase perfusion technique, described previously [14, 15]. Cells were resuspended in buffer containing 120 mM NaCl, 2.6 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 1 mM CaCl₂, 10 mM HEPES, 11 mM Glucose and 2 mM Pyruvate. The experimental protocol for the study of adenosine transport was based on that of Ford and Rovetto [16]. 250 μl of the cell suspension were incubated at 37°C with 50 μl of incubation buffer containing [2,8-³H] adenosine (3 μCi) and [U-¹⁴C] sucrose (0.1 μCi) (I.C.N., High Wycombe, UK) and varying concentrations of extracellular adenosine. Final concentrations of adenosine were 1,10,100 or 1000 μM. Sucrose was present as a marker of the extracellular space. After 1 min incubation, the cell suspensions were rapidly layered on the top of Eppendorf tubes containing 0.1 ml of 2 M perchloric acid covered by a layer of bromododecane and centrifuged to separate cells and medium [17]. As a zero time control, myocyte suspensions were subjected to a similar procedure without 1 min incubation. The time required for collection and layering of the cell suspension to the start of centrifugation was 15 sec. Radioactivity in the cells and the medium was either measured directly by dual label scintillation counting or determined in individual nucleotide metabolite fractions following HPLC separation [18]. Calculations of adenosine transport included a correction for the amount of adenosine present in the medium contaminating the cellular fraction. This correction was less than a few percent at low extracellular concentrations of adenosine but was significant (approximately 20-50%) at 100 and 1000 μM concentrations. Final rates of adenosine transport were determined by subtracting the zero time values from samples incubated for 1 min. Total cellular protein concentration was measured in cell pellets, solubilized in 0.5 M NaOH, using the Lowry assay method [19].

**Enzyme assays**

In a separate series of experiments, hearts from hyperthyroid and euthyroid rats were briefly perfused with 0.9% NaCl to remove blood. Subsequently cardiac tissue was homogenised in ice-cold Tris buffer containing: 150 mM KCl, 20 mM TRIS, 1 mM EDTA, 1 mM Dithiothreitol (pH 7.0) in a ratio of 9 ml of buffer per 1 g of tissue. Crude homogenate was used for the assay of membrane-5'-nucleotidase. For all other enzyme assays, the homogenate was centrifuged at 3700 r.p.m. for 30 min at 4°C and assayed at 37°C as described previously [20-22]. The maximal enzyme activity was calculated from the rate of conversion of substrate into product. The activities measured included adenosine deaminase (ADA), adenosine kinase (AK), AMP-deaminase (AMP-DA), S-adenosylhomocysteine hydrolase (SAHH), purine nucleoside phosphorylase (PNP), xanthine oxidoreductase (XOX), membrane-5'-nucleotidase (M5'N) and cytosolic 5'-nucleotidases: AMP specific (A5'N) and IMP-specific (I5'N).