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Thyroid stimulating hormone directly modulates cardiac electrical activity

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ABSTRACT

Background: The electrocardiogram of hypothyroid patients shows a series of abnormalities of cardiac repolarization due to a reduction of some repolarizing K^+ currents and an increase of the L-type calcium current. Experimental and clinical works call into question the unique role of T3 and T4 in these mechanisms and correlate increased serum TSH levels with the repolarization abnormalities in patients with both subclinical and overt hypothyroidism. In this context, the aim of the present study was to investigate the direct effects of TSH upon cardiac electrical properties.
Methods: The action potential recording and the ion channel subunits mRNA expression were obtained from left ventricle of adult rats. Additionally, the repolarizing K^+ currents and the L-type Ca^{2+} current (ICa-L) were recorded in isolated rat adult ventricular myocytes by the patch-clamp technique.
Results: 24 h exposure to TSH lengthened the action potential and slightly depolarized the resting membrane potential. TSH- receptor activation causes a reduction of the amplitude of I_{to} and I_{K1} currents caused by a reduction in channels expression. However, TSH had no effect on I_{Ca}-L, I_K or I_{Kur}.
Conclusion: These results support the idea that some of the electrical disturbances seen in hypothyroid hearts, such as the I_{to} and I_{K1} current reduction, could be caused not by low T3 but by the elevation of circulating TSH.
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1. Introduction

In the western countries, almost half of the visits to the endocrinologist are related to thyroid dysfunctions. Symptoms specifically related to heart activity are poor exercise tolerance and increased fatigability [1]. In later stages ventricular fibrosis, myocardial edema and reduced cardiac output can be found [2,3,4]. The electrocardiogram of hypothyroid patients shows a series of abnormalities which include sinus bradycardia and cardiac repolarization alterations such as prolonged QT and QTc intervals and higher QT dispersion [3,5,6]. Due to the well-established correlation between repolarization abnormalities and cardiac arrhythmias, the identification of the underlying molecular mechanisms is a matter of great interest. Prolonged repolarization time can be caused by an increase of the depolarizing currents or to a decrease of repolarizing currents. In this sense, several works demonstrated that hypothyroidism reduces some cardiac repolarizing K^+ currents such as the transient outward potassium current (I_{to}) whereas increases the L-type calcium current (ICa-L) [7,8,9,10].

However, previous studies have demonstrated that i) thyroid hormone has no effect on I_{to} current density in adult cardiac myocytes; ii) patients with subclinical hypothyroidism, where T3 and T4 levels are not reduced, share most of the cardiac repolarization abnormalities with overt hypothyroidism [6,11,12]; and iii) several works correlated elevated serum Thyroid Stimulating Hormone (TSH) levels with the repolarization abnormalities in patients with subclinical and overt hypothyroidism [5,11]. Taken together, these experimental and clinical works call into question the unique role of low T3 and T4 levels in these mechanisms, but to date the possible effects of TSH on the cardiac electrical activity has not been explored.

Thus, in the present work we analyzed the direct effects of either acute or 24 h exposure to TSH on the cardiac electrophysiological

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properties. Our results show that although TSH receptor activation does not affect the properties of the L-type calcium current, it directly modulates cardiac action potential duration, and therefore electrical activity, through the regulation of the expression of two potassium currents: the transient outward and the inward rectifier (I_{K1}).

2. Materials and methods

The investigation fulfills the Spanish (RD1201/2005) and European (D2003/65/CE and R2007/526/CE) Guidelines for the Care of Animals Used in Experimental and other Research Purposes, and has been approved by the Ethics Committee for Animal Care of the Universidad del País Vasco (CEBA/46/2010).

2.1. Membrane potential recording

Young adult Sprague–Dawley rats were anesthetized by intraperitoneal injection of chloral hydrate (3 ml/kg). The hearts were removed and perfused with a KB solution (in mmol/l): taurine 10, glutamic acid 70, creatine 0.5, succinic acid 5, dextrose 10, KH_2PO_4 10, KCl 20, HEPES-K^+ 10, EGTA-K^+ 0.2, adjusted to pH 7.4 with KOH. TSH 30 mU/L or vehicle was added to the KB solution to ensure the correct distribution of the drug through the heart. Then epicardial muscle strips were dissected and incubated in KB solution with or without TSH 30 mU/L for 24 h at 4 °C. After incubation, the tissue was pinned to the bottom of a tissue bath in order to expose the epicardial side. The preparations were superfused with Tyrode's solution (in mM): NaCl 150, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, D-glucose 11.0, HEPES 10.0, pH 7.4 adjusted with NaOH. Superfusion was performed at 37 °C saturated with pure oxygen at a constant flow of 5 ml/min (Gilson Minipuls 3). The tissue was stimulated at four different basic cycle lengths (1000 ms, 800 ms, 500 ms and 300 ms). Transmembrane potential was recorded using glass microelectrodes (10–40 MΩ DC resistance) filled with 2.7 M KCl, connected to a high input impedance microelectrode amplifier (Electro 705, World Precision Instruments, USA). Amplified signals were digitized (1440 Digidata A/D interface, Axon Instrument, Inc.) and stored in a computer for later analysis using the software LabChart 7.3 (AD Instruments, Australia). The following action potential (AP) parameters were analyzed: maximum diastolic potential, AP amplitude (APA) and AP duration at 30 and 90% of repolarization (APD_{30} and APD_{90}, respectively). The maximum negative slope was calculated by linear regression as the steepest downhill slope starting 5 ms after the AP peak during a window of 4 ms. The AP triangulation was calculated by subtracting APD_{30} from APD_{90}.

2.2. Cardiomyocyte isolation

Animals were anesthetized by intraperitoneal injection of chloral hydrate (3 ml/kg). The hearts were removed and perfused at 37 °C with: 1) a Tyrode solution containing (in mmol/l): NaCl 118, KCl 5.4, $NaHCO_3$ 24, $MgCl_2$ 1.02, $CaCl_2$ 1.8, NaH_2PO_4 0.42, dextrose 12 and taurine 20, bubbled with 95% O_2 and 5% CO_2, pH 7.4 at 37 °C, 2) the same solution without Ca^{+2}, and 3) the nominally Ca^{2+}-free solution containing collagenase Type I (0.5 mg/ml; CDU ≥ 125 units/mg) and protease Type XIV (0.03 mg/ml; activity ≥3.5 units/mg) and 4) the KB solution. Last, the epicardium of the left ventricle was excised and cardiomyocytes were obtained by mechanical agitation of the ventricle. Aliquots of the KB solution containing the myocyte suspension were incubated for 24 h at 4 °C with TSH or vehicle. In one set of experiments the myocytes were incubated for 2 h with the anti-TSHR antibody (Santa Cruz Biotechnology) and then TSH for 24 h.

2.3. Patch-clamp recordings

Isolated myocytes were transferred to a shallow chamber and allowed to settle for at least 10 min before being superfused with the external bathing solution. For the experiments we used only Ca^{2+}-tolerant rod-shaped cells, with clear cross-striations and lacking any visible blebs on their surfaces. All experiments were performed at room temperature (20–22 °C).

Ionic currents were recorded using the whole-cell configuration of the Patch-Clamp technique with an Axopatch 200B patch-clamp

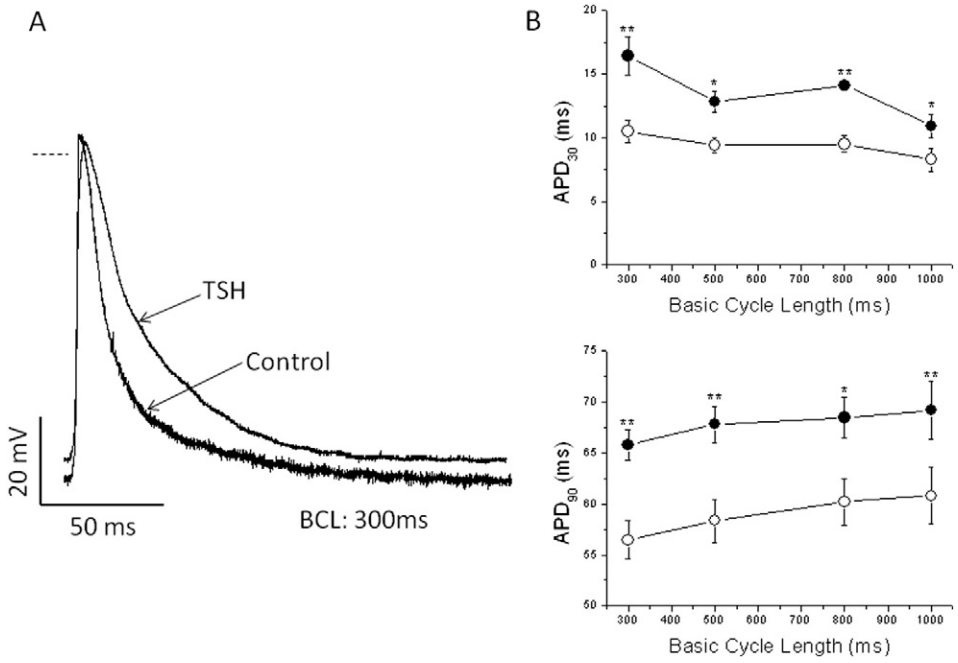

Fig. 1. TSH prolongs ventricular action potential duration. (A) Ventricular action potentials recorded in epicardial strips from the same heart after 24 h incubation with vehicle (control) and TSH 30 mU/L. (B) Action potential duration at 30 and 90% of repolarization (APD_{30} and APD_{90} respectively) at different basic cycle lengths (BCL), after 24 h incubation in control (open symbols) or with TSH (filled symbols). *p < 0.05; **p < 0.01, n = 11 cells from 3 to 5 hearts per group. In this and subsequent figures the dashed line indicates the zero level.

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