Effects of thyroid hormone on action potential and repolarizing currents in rat ventricular myocytes

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Effects of thyroid hormone on action potential and repolarizing currents in rat ventricular myocytes. Am. J. Physiol. Endocrinol. Metab. 278: E302–E307, 2000.—Thyroid hormones play an important role in cardiac electrophysiology through both genomic and nongenomic mechanisms of action. The effects of triiodothyronine (T3) on the electrophysiological properties of ventricular myocytes isolated from euthyroid and hypothyroid rats were studied using whole cell patch clamp techniques. Hypothyroid ventricular myocytes showed significantly prolonged action potential duration (APD90) compared with euthyroid myocytes, APD90 of 151 ± 5 vs. 51 ± 8 ms, respectively. Treatment of hypothyroid ventricular myocytes with T3 (0.1 µM) for 5 min significantly shortened APD by 24% to 115 ± 10 ms. T3 similarly shortened APD in euthyroid ventricular myocytes, but only in the presence of 4-aminopyridine (4-AP), an inhibitor of the transient outward current (Ito), which prolonged the APD by threefold. Transient outward current (Ito) was not affected by the acute application of T3 to either euthyroid or hypothyroid myocytes; however, Ito density was significantly reduced in hypothyroid compared with euthyroid ventricular myocytes.

Acute effects of triiodothyronine; heart; transient outward current; delayed rectifier current.

It is well recognized from studies of patients with spontaneously occurring hypothyroidism and hyperthyroidism that thyroid hormone has profound effects on the cardiovascular system (15). The classically described cellular actions of thyroid hormone are mediated by nuclear triiodothyronine (T3) receptors that function to regulate the expression of specific cardiac genes (4, 8) such as plasma membrane sodium-potassium ATPase (21, 30) and voltage-activated K+ channel genes including Kv4.2, Kv4.3, and Kv1.5 (24, 26, 29). Changes in cardiac electrophysiology have been well documented for chronic thyroid disease states (3, 19, 27, reviewed in Ref. 16). Early experimental studies of thyroid hormone effects on transmembrane potentials of sinoatrial node cells and atrial muscle cells showed an increased rate of diastolic depolarization and decreased duration of action potential in thyrotoxic animals, suggesting that conductance of K+ ions may be altered (11, 14).

Recent evidence has shown that thyroid hormones exert effects on the cardiovascular system that are not mediated by alterations in gene expression (reviewed in Ref. 17). Sakaguchi and co-workers (25) showed that T3 caused a shortening of the action potential duration in guinea pig ventricular myocytes by increasing whole cell inward rectifier potassium current (Ikr). Single Ikr channel recordings showed that T3 increased the open probability mainly resulting from shortening of interburst duration without any changes in burst behavior. Neither the number of channels nor unit amplitude of single Ikr was changed by T3. Sodium channel activity has also been shown to be regulated by T3. Single channel studies by Dudley and Baumgarten (9) showed an increase of burst activity of the sodium channel when T3 was applied extracellularly, whereas Cui and Sen (5) and Harris et al. (12) showed that the increase of sodium current induced by T3 occurred as a result of a slower rate of inactivation of the current.

In the rat ventricular myocyte, two primary depolarization-activated outward currents are important in regulating action potential duration: the Ca2+-dependent transient outward K+ current (Ito) and a slowly inactivating K+ current (Ikr) (1). Although thyroid hormone has been shown to regulate the expression of numerous cardiac-specific genes, the present study was designed to distinguish the genomic and nongenomic effects of T3 on the action potential duration and repolarization currents of ventricular myocytes isolated from both euthyroid and hypothyroid rats.

MATERIALS AND METHODS

Animals. In accordance with institutional animal care committee standards, male Sprague-Dawley rats, each weighing ~170 g, were divided into two groups and treated for 5–6 wk. Group I remained untreated and served as the euthyroid control; group II received 6-propyl-2-thiouracil (PTU) at a concentration of 750 mg/l in the drinking water to induce the hypothyroid state. Heart weights and myocyte size were used to determine the efficacy of the treatment regimen. Myocyte isolation and electrophysiological recordings. Single ventricular myocytes were isolated as previously described (22). The hearts were rapidly removed from anesthetized rats (pentobarbital sodium, 50 mg/kg) and perfused for 5 min in a constant-pressure Langendorff system with standard Ty-
rode's solution (in mM: 10 HEPES pH 7.4, 137 NaCl, 5.4 KCl, 1 MgCl₂, 0.33 Na₂HPO₄, 1.8 CaCl₂, 10 glucose). The perfusate was oxygenated and maintained at 37°C. The hearts were then perfused with Ca²⁺-free Tyrode solution for 5 min followed by the addition of 0.114% (wt/vol) collagenase type I plus 0.014% (wt/vol) protease type XIV (Sigma, St. Louis, MO) for 10 min. The enzyme-containing solution was then washed out by perfusing with Ca²⁺-free Tyrode solution for 5 min, and the ventricles were separated from the rest of the heart. Myocytes were isolated by mechanical agitation in solution containing (in mM): 20 taurine, 50 glutamic acid, 10 HEPES pH 7.2, 0.5 EGTA, 3 MgSO₄, 30 K₂HPO₄, 30 KCl and were used for patch clamp experiments up to 8 h after isolation. The pipette solution containing (in mM) 110 potassium aspartate, 20 KCl, 5 Na₂-ATP, 5.92 MgCl₂, 10 EGTA, and 10 HEPES pH 7.2 was passed through a 0.2-µm sterile filter (Gelman Sciences, Ann Arbor, MI).

Isolated ventricular cells were allowed to adhere for several minutes to a freshly coated polylysine surface in a bath (−200 µl volume) on the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan) and heat polished. When filled with a pipette solution, pipette tip resistance ranged between 2 and 4 MΩ. The liquid junction potential (14.2 mV, pipette negative) was calculated using Axoscope (Axon Instruments) and was zeroed before the formation of the membrane-pipette seal in a standard Tyrode solution. The plasma membrane was ruptured by applying negative pressure in the pipette.

Five-millisecond depolarizing current pulses, sufficient to reach the threshold for sodium current (Iₙa) activation, were used to initiate action potentials using current clamp in whole cell configuration. Action potentials (AP) were recorded at 1 Hz after the APs had reached steady state. Recordings were made from hypothyroid and euthyroid ventricular myocytes in standard Tyrode solution and from euthyroid myocytes in the presence of 4-aminopyridine (4-AP, 3 mM) to inhibit the transient outward current (Iₒ). Triiodothyronine (T₃, 0.1–1.0 µM) was added to the superfusate and action potentials were recorded after 2 min, which represented the earliest measurable response to hormone. The average of ten episodes of action potentials was used for data analysis.

Iₒ was recorded by depolarizing potentials ranging between −30 and +80 mV from a holding potential of −45 mV to inactivate the Na⁺ channels in standard Tyrode solution containing 500 µM CdCl₂ to block the calcium channels. The Iₓ was measured by holding the cells at −45 mV and stepping up to more positive voltages (−30 to +80 mV) for 500 ms. In this protocol, 4-AP (3 mM), BaCl₂ (200 µM), and CdCl₂ (500 µM) were added to the superfusate to inhibit Iₒ, Iₓ, and calcium channels, respectively. After Iₒ or Iₓ measurements were made in myocytes under control conditions, T₃ (10⁻⁶–10⁻⁵ M) was added to the superfusate, and after 2 min the voltage protocol was repeated over a period of ~120 s. Reverse T₃ (rT₃) was tested to determine the specificity of the T₃ effects. Another series of experiments measured the dose-response relationship of Iₓ with T₃ (10⁻⁹–10⁻⁵ M) using a test pulse of +80 mV. Cell capacitance was calculated by integrating the area under the uncompensated capacitance transient produced by 10 mV, hyperpolarizing step from 0 mV, and dividing this area by the voltage step. Currents were digitized at 4 kHz and filtered at 1 kHz. Cell capacitance and pipette series resistance were both compensated. All of the experiments were performed at room temperature (~25°C).

The data were analyzed using pClamp software (Axon Instruments). Iₒ was measured as the peak current at the beginning of the test pulses. Iₓ was measured as the difference between the current at the end of 500-ms pulses and the holding current. The current-voltage relationship was evaluated by plotting the current density (current normalized to the cell capacitance) as a function of the test potentials.

Statistical Analysis. All data are expressed as means ± SE. The statistical significance for any differences was assessed using Student's t-test (paired or unpaired where appropriate).

RESULTS

Animal thyroid status. As a measure of thyroid status, heart weights were measured at the end of the 6-wk treatment period. Hypothyroid hearts were significantly smaller than euthyroid controls, 1,077 ± 20 mg vs. 1,658 ± 10 mg, respectively (P < 0.01; n = 4/group). As a measure of myocyte size, hypothyroid myocyte capacitance (92.5 ± 2.6 pF, n = 22) was significantly reduced (P < 0.05) compared with that of the euthyroid myocyte (158.1 ± 6.1, n = 26). Characteristic of hypothyroidism, the action potential duration was prolonged in ventricular myocytes from the PTU-treated animals.

Effects of T₃ on APD. Action potentials were recorded under whole cell current clamp configuration in ventricular myocytes isolated from euthyroid and hypothyroid rats (Fig. 1). Administration of T₃ (1 µM) to the superfusate had no effect on the APD of euthyroid myocytes (Fig. 1A). The APD at 90% repolarization (APD₉₀) was 52.6 ± 9.4 ms after T₃ addition compared with 51.3 ± 8.3 ms in control conditions without T₃ addition (n = 6). The APD range in euthyroid myocytes was from 30 to 70 ms, as illustrated by the two tracings in Fig. 1A and B. Figure 1B shows that APD₉₀ of euthyroid myocytes could be prolonged to 199.0 ± 16.8 ms (n = 12, P < 0.05) in the presence of 4-AP (3 mM), which blocks the Iₒ. The addition of T₃ to the superfusate caused a significant shortening of APD₉₀ in the presence of 4-AP to 175.9 ± 14.9 ms (n = 12, P < 0.05), suggesting that T₃ had an effect on a 4-AP insensitive current. Figure 1C shows that the APD₉₀ in the hypothyroid myocyte was prolonged (151 ± 4.6 ms, n = 6) and that the addition of T₃ (0.1 µM) significantly shortened (P < 0.05) the APD₉₀ to 115.3 ± 10.2 ms.

Effects of T₃ on ionic currents. To determine the mechanisms underlying the rapid effects of thyroid hormone on ventricular myocyte action potentials, the two predominant depolarization-induced potassium currents, Iₒ and Iₓ, were examined in a series of experiments using whole cell voltage clamp techniques. The rapidly inactivating Iₒ can be blocked by 4-AP, whereas the 4-AP-insensitive or delayed rectifier current (Iₓ) can be blocked by high concentrations of tetraethylammonium (TEA) (1).

The Iₒ was recorded in the presence of CdCl₂ using 500-ms depolarizing pulses from a holding potential of −45 mV as shown in Fig. 2. Figure 2A shows Iₒ current

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traces in a single euthyroid myocyte before and after the addition of T3 (1 µM) to the superfusate, and Fig. 2B shows similar traces in a hypothyroid myocyte. No effect of T3 on Ito in either euthyroid or hypothyroid myocytes was observed; however, Ito amplitude was significantly reduced (P < 0.05) in the hypothyroid compared with the euthyroid myocyte and from 140 to 160 ms in hypothyroid myocytes.

The I_K was recorded by depolarizing pulses ranging from −30 to +80 mV for 500 ms from a holding potential of −45 mV in the presence of CdCl2, 4-AP, and BaCl2. Figure 3 shows typical current traces recorded before and after administration of T3 (0.1 and 1.0 µM) in euthyroid (A) and hypothyroid (B) ventricular myocytes. The I_K densities in euthyroid and hypothyroid myocytes are plotted against the test potentials, showing no differences between the two conditions when measured under control conditions without T3 superfusion. However, in response to 1 µM T3, significant increases in I_K were observed at test potentials more positive than +20 mV and +10 mV in euthyroid and hypothyroid myocytes, respectively. At a lower concentration of T3 (0.1 µM), a significant increase in I_K was observed at test potentials more positive than +20 mV (P < 0.05) in the hypothyroid myocyte compared with +70 mV in the euthyroid condition, suggesting a higher sensitivity or responsiveness to T3 in the hypothyroid myocyte.

Figure 4 shows in greater detail the I_K current in a euthyroid myocyte in response to T3 to illustrate that both the activation and the inactivation properties of the current were altered by the hormone. A slower rate of activation was observed at +70 mV in the presence of T3 with an increase of the time-to-peak I_K of 69 ± 6 ms compared with 44 ± 7 ms without T3 (n = 6, P < 0.05). Although not shown in this figure, the inactivation time constants were derived from a series of experiments using a test step duration of 3 s. Curve-fitting by

\[ I_{\text{to}} \text{ (pA/pF)} = \text{plotted as a function of test pulse voltage in euthyroid and hypothyroid myocytes with and without T3 treatment (C). P < 0.05 between euthyroid vs hypothyroid; n = 8.} \]
monoexponential function to the first 1,500 ms of current traces showed that T3 (1 µM) increased the time constant of inactivation (at +70 mV) from 353 ± 28 to 420 ± 31 ms (n = 5; P < 0.05). The amplitude of this exponential component was unchanged by T3 (420.5 ± 91.9 vs. 474.3 ± 95.0 pA).

To determine the specificity of the T3 effect on I_K, the effects of the biologically inactive metabolite rT3 were examined. Current density measurements as a function of test voltages before and after rT3 addition showed no effect on I_K in either hypothyroid or euthyroid myocytes (Fig. 5). These data support the specificity of the observed effects on myocyte ion currents to the biologically active hormone T3.

Dose responsiveness of T3 on I_K. Concentration dependence of T3 on I_K current density in both euthyroid and hypothyroid myocytes is shown in Fig. 6. The current magnitudes measured at the end of a 500-ms test pulse of +80 mV were normalized by the amplitude of I_K under control conditions in each experiment and analyzed as a function of T3 concentration added to the superfusate. The increase of I_K by T3 in hypothyroid myocytes was significantly greater than in the euthyroid myocyte at 10⁻² and 10⁻³ M concentrations, indicating a greater sensitivity to T3 in the hypothyroid condition.

**DISCUSSION**

The present study has shown that the APD of ventricular myocytes isolated from hypothyroid rats and 4-AP-treated myocytes from euthyroid animals can be shortened by acute exposure to T3, at least in part by increasing the I_K. Superfusion of the hypothyroid myocyte with T3 shortened the APD by 24%; however, this did not restore the APD to euthyroid values and can be attributed to a primary role of the I_to on the APD in the rat myocyte. The transient outward K⁺ current was significantly lower in the hypothyroid myocyte and was not altered by acute exposure to T3. Our findings are somewhat in contrast to a report by Sakaguchi et al. (25), who showed that T3 shortened APD in euthyroid guinea pig ventricular myocytes in the absence of 4-AP treatment. This difference in results may reflect the longer APD₉₀ in the euthyroid guinea pig compared with the rat myocyte (647 ± 13 vs. 51 ± 8 ms, respectively), reflecting species differences.
The rapidity of the T3 effect on IK suggests that the mechanism by which T3 acts does not involve changes in the expression of specific T3-responsive cardiac genes because this would require a finite period of time for transcription, RNA processing, and protein synthesis before the manifestation of a biological effect (4, 8). Nonnuclear effects of T3 that are rapid in onset have been previously reported to change adrenergic receptor responses in the heart, sarcolemmal Ca2+-ATPase activity, the inward rectifier IK1 channel, and sodium channels (5, 9, 12, 25, reviewed in Ref. 6). Because we have recently shown that the content of Kv1.5 mRNA (half-time, 2 h) in ventricles of hypothyroid rats was increased to euthyroid levels within 1 h of T3 treatment in vivo (24), the present studies were conducted to record the earliest electrophysiological responses to T3. Significant effects on IK in this study were observed within 5 min of T3 exposure, whereas a significant effect on IK1 was not reported until 15 min after superfusion with T3 (25).

In contrast to these rapid T3 effects, the current density of Ito was significantly reduced in hypothyroid compared with euthyroid ventricular myocytes, suggesting that the molecular components of Ito that include the Kv4.2 and Kv4.3 gene products are regulated by thyroid hormone at the transcriptional level (26). The reduction in density of the Ito in chronic hypothyroidism, as shown in the present study and by others (27), can partially explain the prolongation of APD and the prolonged Q-T interval observed in electrocardiogram tracings. Contrary to the rapid effects of T3 on IK, addition of T3 to isolated ventricular myocytes from either euthyroid or hypothyroid animals had no effect on the ionic characteristics of Ito, further supporting the observation that the rapid-onset effects of T3 are distinct from its transcriptional effects and are selective to specific ion channels.

Potential molecular mechanisms by which T3 may elicit the observed rapid effects on ion flux may involve direct binding of T3 to either an intracellular or extracellular site on the IK channel, or alternatively, that T3 triggers intracellular signaling events that regulate the IK channel gating activity. Dudley and Baumgarten (9) used patch excision methodology to show that the rapid (within 30 s) effects of T3 on Na+ channel gating required exposure of the hormone to the extracellular face of the Na+ channel and that soluble second messengers were unlikely to be involved. The voltage clamp protocol in the whole cell configuration used in the present study is unable to distinguish among these possibilities.

The mechanism for the increased sensitivity of IK to T3 in the hypothyroid compared with the euthyroid myocyte is not known. It is possible that expression of functionally different isoforms of IK channel subunits may underlie the changes in the sensitivity to T3 or alternatively, that the intracellular signaling pathways may be different between the two conditions. Recently, several studies have shown that protein kinases are involved in regulating specific potassium channels (2, 3).
13, 22). Published data have also indicated that T₃ activates protein kinase C and cAMP-dependent protein kinase signaling pathways in other cell types (20) and modulates sarcoplasmic reticulum calcium transients in cardiac cells (reviewed in Refs. 6, 7). These results have been used to explain the rapid inotropic effect of T₃ on cardiac myocytes (28) and on enhanced systolic function in patients with varying degrees of overt and subclinical hypothyroidism. These results have important implications in the treatment of ventricular arrhythmias in patients with varying degrees of overt and subclinical hypothyroidism.

In summary, our data show that T₃ shortens the APD in hypothyroid rats due at least in part to the increase in the delayed rectifier current Iₖ. The Iₖo appears to be regulated by thyroid hormone at the transcriptional level, whereas the Iₖs is regulated by a nongenomic mechanism of action. These results have important implications in the treatment of ventricular arrhythmias in patients with varying degrees of overt and subclinical hypothyroidism.

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