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 (Iₒ). Single Iₒ 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 Iₒ 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 in 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⁺-independent transient outward K⁺ current (Iₒ) and a slowly inactivating K⁺ current (Iₚ) (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-mercaptopurine (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 KH₂PO₄, 30 KCl and was 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 hypertrophied ventricular myocytes in standard Tyrode solution and from euthyroid ventricular myocytes in the presence of 4-aminopyridine (4-AP, 3 mM) to inhibit the transient outward current (Iₒ). Triiodo-L-thyronine (T₃, 0.1–1.0 µM) was added to the superfusate, and after 2 min the membrane-pipette seal was ruptured by applying negative pressure in the pipette.

Figure 1 shows that the APD 90 in the hypothyroid ventricular myocyte was prolonged (151.1 ± 6.8 ms in control conditions without T3, 6n) 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 ventricular 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 Iₓ 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 Iₓ current...
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 Iᵯ in either euthyroid or hypothyroid myocytes was observed; however, Iᵯ amplitude was significantly reduced (P < 0.05) in the hypothyroid compared with the euthyroid myocyte. Figure 2C shows a summary of the data of peak Iᵯ density as a function of test potentials in response to T₃ from an average of eight euthyroid and hypothyroid myocytes.

The Iᵯ 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 Iᵯ 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 Iᵯ 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 Iᵯ 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 Iᵯ 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 Iᵯ 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
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 IK, 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 IK 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 IK. Concentration dependence of T3 on IK 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 IK under control conditions in each experiment and analyzed as a function of T3 concentration added to the superfusate. The increase of IK 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 IK. 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 Ito 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 I_K 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 I_K₁ 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 I_K in this study were observed within 5 min of T₃ exposure, whereas a significant effect on I_K₁ was not reported until 15 min after superfusion with T₃ (25).

In contrast to these rapid T₃ effects, the current density of I_to was significantly reduced in hypothyroid compared with euthyroid ventricular myocytes, suggesting that the molecular components of I_to 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 I_to 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 I_K, addition of T₃ to isolated ventricular myocytes from either euthyroid or hypothyroid animals had no effect on the ionic characteristics of I_to 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 I_K channel, or alternatively, that T₃ triggers intracellular signaling events that regulate the I_K 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 I_K to T₃ in the hypothyroid compared with the euthyroid myocyte is not known. It is possible that expression of functionally different isoforms of I_K 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, 5).

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FIG. 5. Concentration-response relationship of T₃-induced increase of I_K in euthyroid and hypothyroid ventricular myocytes. T₃-induced increase in I_K amplitude measured at end of a 500-ms test pulse of +80 mV was normalized by the amplitude of I_K under control conditions. The increase of I_K by T₃ in hypothyroid myocytes was significantly greater than that in euthyroid myocyte at 10⁻⁷ and 10⁻⁸ M T₃ (*P < 0.05).
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 cardiac contractility in vivo using an isolated perfused heart preparation (18). Our previously published data have shown the presence of high-affinity T₃-binding sites on the plasma membrane of vascular smooth muscle cells that may be necessary for the rapid vasodilatory effects of T₃ (23).

In summary, our data show that T₃ shortens the APD in hypothyroid rats due at least in part to the increase of the delayed rectifier current Iₖ. The Iₖ appears to be regulated by thyroid hormone at the transcriptional level, whereas the Iₖ 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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