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HYPERTHYROIDISM SELECTIVELY MODIFIED A TRANSIENT POTASSIUM CURRENT IN RABBIT VENTRICULAR AND ATRIAL MYOCYTES

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SUMMARY

1. Transient outward potassium currents (I_t) were compared in single cardiac myocytes obtained from normal and hyperthyroid rabbits. Currents were recorded using the suction electrode whole-cell voltage clamp technique.

2. In ventricular myocytes from hyperthyroid animals (at 22 °C and a stimulation rate of 0·2 Hz), I_t was 4- to 5-fold larger than in normal myocytes, in a potential range of −20 to +60 mV. As in normal myocytes, I_t in hyperthyroid myocytes was calcium insensitive, and was more than 90% suppressed by 2 mM 4-aminopyridine.

3. The increase in I_t was observed over a wide range of stimulation rates, even at rates sufficiently slow to enable complete reactivation of the I_t channels. However, there was a major change in the rate dependence of I_t in hyperthyroid myocytes, with significant I_t current still present at rates (e.g. 1–2 Hz) at which it is normally completely suppressed.

4. The augmentation of I_t in the hyperthyroid myocytes could not be accounted for by changes in the voltage dependence or the kinetics of channel activation or inactivation. There was no change in the reversal potential of I_t, implying no change in the selectivity of the channel.

5. Single-channel activity was recorded using the cell-attached mode of recording. In myocytes from hyperthyroid rabbit we observed the following: (a) active patches (often containing two channels) were obtained more frequently in comparison to control; (b) the unitary conductance of the channel was the same; (c) single-channel openings persisted at high stimulation rates.

6. In contrast to hyperthyroid *ventricular* cells, I_t in *atrial* cells from the same hearts was not substantially changed.

7. The rate dependence of I_t in atrial cells was also unaffected by hyperthyroidism, in contrast to the large changes observed in ventricular cells. Thus, in atrial cells from hyperthyroid hearts the current was totally suppressed at rates of 1–2 Hz, as in euthyroid conditions.

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8. Single-channel recordings in the cell-attached mode showed a unitary conductance similar to that found in normal atrial cells. Channel activity was suppressed at 2 Hz, in contrast to hyperthyroid ventricular cells.

9. In conclusion, I_t is drastically changed in hyperthyroid rabbit ventricle cells. The changes are in the magnitude of the macroscopic current and its rate dependence. Since the unitary conductance is unchanged (and the peak open probabilities are normally high at positive membrane potentials) the number of active channels in the membrane must be increased. In atrial cells from the same hyperthyroid hearts no changes are apparent. These results are discussed in terms of the functional implications for action potential configuration, and in terms of the changes which occur in myosin isozyme pattern changes in the two cardiac tissues. This is related to the issue of long-term cardiac electrical and mechanical adaptation.

INTRODUCTION

The hyperthyroid heart has been well characterized (Buccino, Spann, Pool, Sonnenblick & Braunwald, 1967; Morkin, Flink & Goldman, 1983; Sharp, Neel & Parsons, 1985; Josephson, Spurgeon & Lakatta, 1990). Cardiac performance is significantly altered, with an increase in heart rate and in the force and velocity of contraction, with concomitant hypertrophy (Morkin *et al.* 1983). The nature of many of these adaptations has been clarified by measuring the mechanical, electrophysiological, and biochemical properties of hyperthyroid hearts. More recently, direct measurements of intracellular calcium transients (MacKinnon & Morgan, 1986) and of transmembrane ionic currents (Rubinstein & Binah, 1989) have further characterized some of the cellular changes in the hyperthyroid state.

Some of the mechanisms underlying the actions of thyroid hormone have been elucidated. Thus, it is now known that the hormone binds to nuclear receptors (Oppenheimer, 1979) triggering the specific expression of several genes (Everett, Sinha, Umeda, Jakovcic, Rabinowitz & Zak, 1984; Lompre, Nadal-Ginard & Mahdavi, 1984; Samuels, Forman, Horowitz & Ye, 1988). Overall, protein synthesis is enhanced (Michels, Cason & Sokoloff, 1963), with an increase in oxidative metabolism and glycolytic capacity (Seymour, Eldar & Radda, 1990). The improvement in cardiac performance is largely due to shifts in myosin isoform distribution, so that the V_1 form predominates (Dillmann, 1984; Everett *et al.* 1984). The faster ATPase action of this isoform underlies the enhanced speed of contraction. Many transport mechanism are also enhanced, such as the sarcoplasmic reticulum calcium pump (Suko, 1973) and the sarcolemmal sodium–potassium pump (Kim & Smith, 1984).

Changes in electrical activity in hyperthyroid conditions have been well defined (Morkin *et al.* 1983; Sharp *et al.* 1985; Binah, Rubinstein & Gilat, 1987). Studies investigating underlying ionic mechanism, using voltage clamp methods, have concentrated on calcium currents. Previous reports suggested complex changes in calcium channel function in hyperthyroid animals, with either a decrease in channel density (in the rat ventricle – Hawthorn, Gengo, Wei, Rutledge, Moran, Gallant & Triggle, 1988; Kosinski, Gross & Hanft, 1990), or an enhanced influx in chick or guinea-pig myocytes (Kim, Smith & Marsh, 1987; Rubinstein & Binah, 1989). Only

one group has studied outward currents, finding an increase in outward potassium current (I_K) in guinea-pig myocytes (Rubinstein & Binah, 1989).

In the rabbit ventricle, Sharp *et al.* (1985) reported complex changes in the action potential configuration, suggesting changes in the major time-dependent outward current in this tissue, the transient outward potassium current, I_t (Giles & Imaizumi, 1988; Clark, Giles & Imaizumi, 1988).

The aim of the present study was 2-fold. Firstly, to study in detail the effects of hyperthyroidism on I_t in rabbit ventricular cells. This was done in the context of investigating long-term cardiac adaptation to changing demands, such as occur during some types of hormonal imbalance. It was of great interest to establish whether, and in what manner, electrical and mechanical parameters (such as myosin isozyme distribution) change in parallel under conditions of stress or altered metabolic status.

The specific rationale for the experiments was the following: at the very high heart rates observed under hyperthyroid conditions, the action potential duration shortens (e.g. Sharp *et al.* 1985). This is essential, in order to enable a sufficient interval between action potentials, in which refilling of the heart can take place. The ionic currents underlying the action potential must therefore alter, to produce the shorter action potential duration.

The second major aim of this work relates to an intriguing feature of cardiac adaptation to hyperthyroidism. Despite the identity of myosin isozymes in atrial and ventricular tissues (Chizzonite, Everett, Prior & Zak, 1984; Izumo, Nadal-Ginard & Mahdavi, 1986) there is no shift in myosin isoforms in atrial tissue in response to elevated thyroxine levels (Banerjee, 1983; Nadal-Ginard & Mahdavi, 1989), although in ventricular tissue this is one of the most potent stimulii for such shifts (Dillmann, 1984). We therefore set out to investigate whether the unresponsiveness of atrial tissues to thyroid hormone elevation was evident in electrical parameters (ionic currents) as well as in mechanical parameters (myosin shifts, contractile efficiency).

Some preliminary results were reported in brief earlier (Shimoni & Banno, 1991).

METHODS

Cell preparation

Isolated myocytes were prepared using a standard enzymatic dispersion method. Male or female New Zealand White rabbits (1·5–2·5 kg) were anaesthetized with sodium pentobarbitone (20 mg kg^{-1}, I.V.), and killed by cervical dislocation. The hearts were rapidly removed and mounted on a Langendorff perfusion apparatus. The heart was perfused retrogradely through the aorta, (at 80 cmH_{2O}, 37 °C) for 5 min in a Tyrode solution containing (mM): NaCl, 121; KCl, 5·0; sodium acetate, 2·8; Na_2HPO_4, 5·0; $MgCl_2$, 1·0; $CaCl_2$, 2·2; $NaHCO_2$, 24; glucose, 5·0, bubbled with a 95% O_2–5% CO_2 mixture. The perfusion was then changed to a low-calcium Tyrode solution (5 μM calcium) for 10 min, and subsequently to Tyrode solution containing 10 units ml^{-1} collagenase (Yakult Honsha, Tokyo) and 10 mg ml^{-1} protease (Sigma, type XIV), as well as 20 mM taurine. After 10 min, chunks were removed from the right ventricle and placed in Tyrode solution containing 100 μM $CaCl_2$, 20 mM taurine and 10 mg ml^{-1} albumin. Aliquots were then removed sequentially for a period of up to 15 min, and cells were stored in a Tyrode solution containing 150 μM $CaCl_2$, 20 mM taurine and 10 mg ml^{-1} albumin. For current and action potential recordings, the cells were perfused with Tyrode solution containing (mM): NaCl, 150; KCl, 5·4; $CaCl_2$, 2·2; $MgCl_2$, 1·0; HEPES, 5·0; glucose, 5·5, brought to a pH of 7·4 with NaOH. The perfusate was bubbled with 100% O_2. To isolate I_t, 0·5 mM $CdCl_2$ was added to block calcium currents. In some

experiments 25 μM tetrodotoxin (TTX) was also used to block sodium currents. This did not alter any measurements of I_t. The experiments were carried out at 22 °C.

Atrial cells

These were prepared in an identical manner, except that at the end of the enzymatic perfusion the atria were removed and cut up into chunks which were placed in a beaker containing 0·5 mg ml^{-2} collagenase and 0·5 mg ml^{-1} protease. Aliquots were removed into the storage solution over the next 5–15 min.

Current recordings

Single myocytes were voltage clamped using the whole-cell suction microelectrode method (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). An EPC 7 amplifier (List-electronic, Darmstadt, FRG) was used. The data were digitized at a sample rate of 1 kHz, after being filtered (4-pole Bessel) −3 dB at 3 kHz. The electrodes (1·5–3·0 MΩ resistance, WPI glass, 1·5 mM o.d., WPI Instruments, New Haven, CO, USA) contained (mM): potassium aspartate, 120; KCl, 30; Na_2ATP, 4; HEPES, 5·0; $MgCl_2$ 1·0, buffered to 7·2 with KOH.

I_t amplitudes were measured from the peak outward to the steady-state level at the end of the depolarizing pulse. In addition, it should be noted that by using aspartate in the pipette filling solution, the measured membrane potential was offset by 10 mV (in the positive direction) due to a liquid junction potential. This was not corrected for in the plots shown (except Fig. 1), but has been pointed out in several places in the Results section (e.g. half-activation/inactivation potentials, reversal potential determination). The cell capacity was measured routinely by integrating capacitative currents recorded with a 25 kHz digitizing sampling rate. The mean ventricular cell capacity was 60·4 ± 1·7 pF(± S.E.M.) in euthyroid (fifty-six cells), and 75·4 ± 2·9 pF in thirty-seven hyperthyroid myocytes. The increase in capacity is presumably due to the increase in cell dimensions which occurs during thyroxine-induced hypertrophy (Morkin *et al.* 1983). Currents are usually represented in terms of density by dividing peak amplitudes by the cell capacity. This normalization minimizes differences due to changes in cell dimensions.

Single-channel recordings

These were made (at 22 °C) by using fire-polished electrodes (1·5–3·0 MΩ resistance), coated with Sylgard. The pipette filling solution was the normal Tyrode solution. Currents were recorded at a sampling rate of 10 Hz after filtering −3dB (4-pole Bessel) at 1 or 2 kHz. After formation of tight gigaseals by applying negative pressure, each patch was tested for the presence of sodium currents, by giving depolarizing steps to 20–30 mV positive to the resting potential. The presence of sodium currents served as an indication that the patch and cell were viable. I_t channel activity was elicited by giving depolarizing pulses from a holding potential of −50 mV with respect to rest, to a range of potentials between 50 and 125 mV positive to the resting potential. Pulses were applied at 0·2 or 2 Hz. Blank traces were averaged and used for substraction of leakage and capacity currents. Current amplitudes were measured manually from computer-generated plots of individual traces (a comparison to measurements using computer software gave virtually the same amplitudes). At least twenty-five channel openings were averaged to obtain mean amplitudes. Current amplitudes were plotted against membrane potential to obtain current–voltage relationships.

Induction of hyperthyroidism

Rabbits were given daily intraperitoneal injections of thyroxine (0·4 mg kg^{-1}) for 5–7 days. Plasma thyroxine levels were measured by standard radioimmunoassay, giving mean levels of 36 ± 5·5 nmol l^{-1} (n = 6) for normal and 683 ± 90 nmol l^{-1} (n = 7) in the hyperthyroid animals. The terms normal and euthyroid are interchanged throughout the text.

RESULTS

Action potentials

The initial set of experiments were designed to establish whether the earlier observations of Sharp *et al.* (1985), done in a multicellular rabbit ventricle preparation, could be reproduced in single myocytes. These have the advantage of

bypassing potential complications inherent in multicellular recordings such as alterations in cell connectivity, in propagation, or in ionic concentration changes in intercellular clefts. In single ventricular myocytes obtained from hyperthyroid animals, no changes were observed in the resting membrane potentials: the values (corrected for junction potentials) at 22 °C were $-76{\cdot}1 \pm 0{\cdot}5$ mV for euthyroid cells ($n = 40$), and $-77{\cdot}7 \pm 0{\cdot}7$ mV for hyperthyroid cells ($n = 25$). However, as found by Sharp *et al.* in multicellular tissue, the action potentials were quite different. At low rates of stimulation, an initial rapid repolarization was followed by a long plateau, very often exhibiting a secondary 'hump' of depolarization, as shown in Fig. 1*A*. This configuration resembles the action potentials recorded by Sharp *et al.* (1985), as well as by Litovski & Antzelevitch (1988) in canine epicardial tissue, and strongly suggests a major role for the transient outward potassium current I_t (as proposed by Sharp *et al.* 1985). Further evidence for a role of this current in producing these complex action potential configurations was obtained by applying 4-aminopyridine (4-AP) or increasing the rate of stimulation. Both of these procedures, which are known to reduce I_t, also changed the action potential shape recorded in the hyperthyroid myocytes to a more 'normal' shape (Fig. 1*A* and *B*). However, an important feature should be pointed out. At high rates of stimulation, I_t is normally suppressed. One would therefore expect little additional effect of 4-AP at the higher rates. Nevertheless, in the hyperthyroid myocytes, even at the higher rates there was still an additional prolongation of the action potential after exposure to 4-AP (Fig. 1*B*). This suggests that I_t is not only larger, but also different in some of its characteristics (see below).

I_t *magnitude*

Whole-cell recordings of I_t (with calcium currents blocked by $CdCl_2$) confirmed that the magnitude of I_t is greatly augmented in hyperthyroid cells. Figure 2*A* (shows sample current traces from typical normal and hyperthyroid myocytes, illustrating the larger currents at all membrane potentials. Figure 2*B* also shows the mean current–voltage relationship for I_t in normal ($n = 28$) and hyperthyroid ($n = 26$) myocytes. Mean ($\pm$S.E.M.) current densities (pA pF^{-1}) are plotted against membrane potential, showing the augmentation to be present at all voltages. The threshold for current activation (the membrane potential at which current was first measurable) was unchanged.

I_t *pharmacology*

A variety of transient outward currents have been described, in rabbit and other cardiac tissues (Kenyon & Gibbons, 1979; Siegelbaum & Tsien, 1980; Boyett, 1981; Escande, Coulombe, Faivre, Deroubaix & Coraboeuf, 1987; Hiraoka & Kawano, 1989; Zygmunt & Gibbons, 1991). Normally, I_t in rabbit ventricle is calcium independent and 4-AP sensitive. It was important to consider whether hyperthyroidism induced the appearance of a different type of I_t in these cells. A calcium-dependent transient outward current (Hiraoka & Kawano, 1989; Zygmunt & Gibbons, 1991) would not be evident in the presence of $CdCl_2$. However, it was also important to rule out the induction of a calcium-independent, 4-AP-resistant transient outward current, such as described by Apkon & Nerbonne (1991). It was

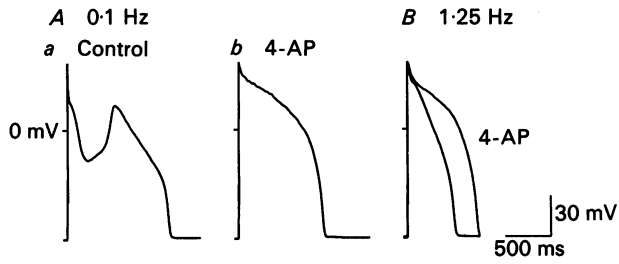

Fig. 1. Action potentials recorded from a hyperthyroid myocyte at 22 °C. At a low rate of 0·1 Hz (*Aa*), a typical complex configuration is shown, with a secondary 'hump' following an initial rapid repolarization. This can be abolished by 2 mM 4-aminopyridine (*Ab*, at 0·1 Hz) or by increasing the rate of stimulation, e.g. to 1·25 Hz (*B*). Even at this high rate, 4-AP is still effective, producing a substantial prolongation, as shown by the superimposed trace. These records suggest a large I_t which is still present at high stimulation rates (see text).

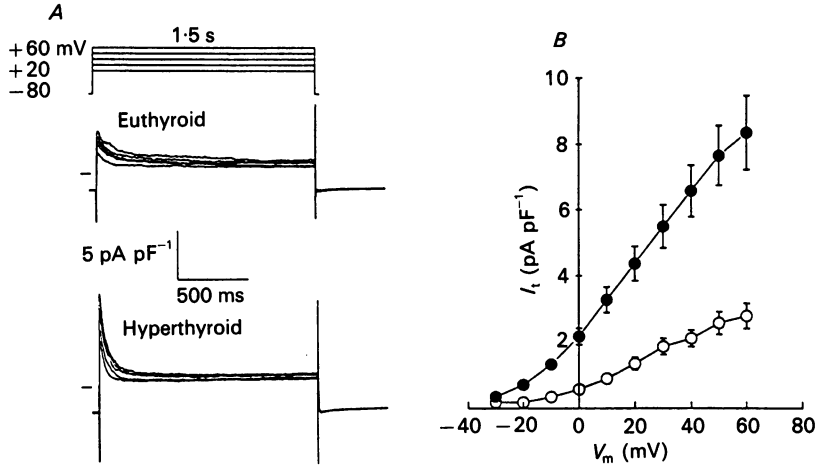

Fig. 2. Transient outward currents recorded from euthyroid and hyperthyroid myocytes, at 22 °C. The cells were held at −80 mV and 1·5 s pulses were given at a rate of 0·2 Hz, to potentials between −30 and +60 mV. *A*, superimposed current traces obtained in response to pulses to +20, +30, +40, +50 and +60 mV in a euthyroid (top) and hyperthyroid cell (bottom). *B*, current–voltage relationship obtained by plotting the mean current densities (pA pF^{-1}) for 9–28 euthyroid (○) and 17–26 hyperthyroid (●) cells against membrane potential (V_m). Standard error bars are also shown. A large increase in current density is apparent at all potentials, with no obvious shift in the threshold for activation. $CdCl_2$ (0·5 mM) present throughout.

found that I_t in hyperthyroid myocytes, although much enhanced, is still sensitive to 4-AP. Thus, the combination of 0·5 mM Cd^{2+} and 2 mM 4-AP eliminates more than 90% of the current ($n = 4$, results not shown). The predominant (if not the sole) component of the transient outward current in these myocytes is 4-AP sensitive and is not calcium activated.

There are several mechanisms by which I_t could be enhanced in the hyperthyroid state. One important consideration is based on the large rate sensitivity of the current (Giles & Imaizumi, 1988). Thus, it is conceivable that current magnitudes are

larger in hyperthyroid cells only because at 0·2 Hz more current is available, in comparison to control. Furthermore, the I_t channel gating kinetics could be altered, as could the voltage dependence of channel gating. In addition, the channel selectivity or conductance could be changed. These possibilities were examined.

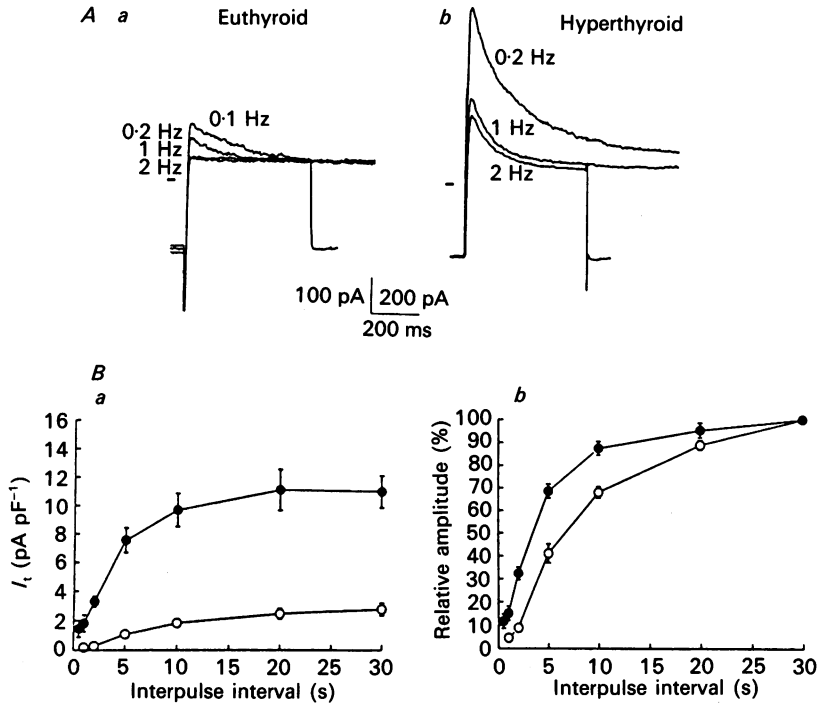

Fig. 3. The rate dependence of I_t in euthyroid and hyperthyroid myocytes. *A*, superimposed steady-state current records obtained at stimulation rates of 0·1, 0·2, 1 and 2 Hz in a euthyroid cell (*Aa*), and at 0·2, 1 and 2 Hz for a hyperthyroid cell. (*Ab*). The currents in the cell from the hyperthyroid rabbit persist at the high rates, whereas in normal cells I_t is completely suppressed at 1 Hz. *Ba*, plot of the mean steady-state amplitudes of I_t at different rates (interpulse intervals) for euthyroid (○) and hyperthyroid (●) cells. Even at the longest interpulse interval (30 s), when the current is fully available, I_t is dramatically increased in the hyperthyroid cells. *Bb*, plot of the relative currents (normalized as 100% at 0·033 Hz), at different intervals. In the hyperthyroid cells, the relative amount of current is larger for most of the range of intervals, indicating that the current reactivates at a faster rate.

Rate dependence of I_t

I_t is known to be extremely rate sensitive (Boyett, 1981; Clark *et al.* 1988). It was therefore possible that a major change occurs in the hyperthyroid state, so that a spurious increase was observed at our basal stimulation rate of 0·2 Hz (Fig. 2). The next series of experiments compared the rate dependence of I_t in normal ($n = 8$) and hyperthyroid ($n = 7$) myocytes. Two important results were obtained from this comparison. First, it was found that I_t was greatly enhanced over a wide range of interpulse intervals, ranging from 500 ms (2 Hz) to 30 s (0·033 Hz). Figure 3*A* shows these results, illustrating that even at the slowest rate (longest interpulse interval),

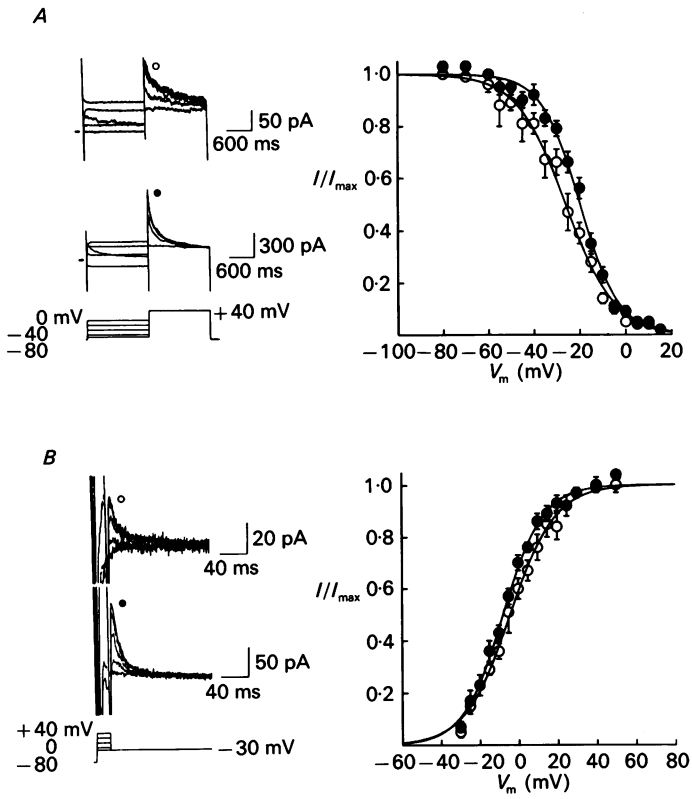

Fig. 4. *A*, steady-state voltage dependence of inactivation of I_t in euthyroid and hyperthyroid myocytes. Inactivation was measured by a standard two-pulse protocol (shown on left), with a prepulse to varying potentials (given at 0·1 Hz), followed by a test potential (+40 mV). The mean amplitudes of I_t (with standard error bars), divided by the maximal amplitude, are plotted as a function of the prepulse potential for the euthyroid (○) and hyperthyroid cells (●). A Boltzmann curve was fitted to the data, using the equation: $I/I_{max} = 1/(1+\exp((V_m - V_{\frac{1}{2}}/S))$. The best fit was obtained with $V_{\frac{1}{2}}$ values of −26·0 and −19·7 mV for euthyroid and hyperthyroid cells, respectively (uncorrected for junction potentials – see text). The slope factors S were 10·7 and 8·4 mV, respectively. A slight depolarizatory shift can be seen in the hyperthyroid cells (see text). *B*, steady-state voltage dependence of activation of I_t in euthyroid and hyperthyroid cells. Activation was measured by analysis of 'tail' currents obtained after brief activating pulses (20 ms) to potentials between −30 and +40 mV (from −80 mV, once every 10 s). These prepulses were followed by a second pulse to −30 mV (protocol shown schematically on the left). The 'tail' current amplitude reflects the degree of activation elicited by the prepulse. The superimposed curves were obtained by plotting the normalized mean (with standard errors) current values (tail amplitudes divided by the maximal amplitude), against the activating potential (○, euthyroid; ●, hyperthyroid). A Boltzmann curve ($I/I_{max} = 1/(1+\exp((V_m - V_{\frac{1}{2}})/S))$) was fitted to the data, with the best fits giving a half-activation voltage of −4·2 and −7·8 mV (uncorrected for junction potentials) for the control and hyperthyroid cells, respectively. The slope factors were −11·8 and −10·4 mV, respectively.

when the current is fully available, there is still a large increase in I_t magnitude. Thus, this enhancement cannot be entirely due to changes in the rate dependence of I_t. However, if the relative current amplitudes are plotted against the interpulse

interval (by normalizing all amplitudes relative to the fully available current, at 0·033 Hz), a significant enhancement of the repriming of the current can be seen, as shown in Fig. 3*B*.

Thus, at the higher rates, the relative amount of available current is considerably larger than in normal myocytes. In fact, in the hyperthyroid myocytes, I_t can be measured at rates (1–2 Hz) at which it is normally completely suppressed, as illustrated in Fig. 3*Ab*.

Activation and inactivation voltage dependence and kinetics

The voltage dependence of the steady-state activation and inactivation parameters overlaps (Clark *et al.* 1988). At the overlapping membrane potentials, which correspond to the plateau phase of the action potential, some steady-state 'window' current can contribute to the action potential. During the action potential plateau, all currents are small so that even small changes can have great impact on the repolarization of the action potential. Thus, in the hyperthyroid state, changes in the 'window' current may be of great relevance. This issue was addressed by studying the voltage dependence of the steady-state activation and inactivation parameters, using standard two-pulse protocols (Giles & Imazumi, 1988). The activation parameter was assessed from analysis of tail currents at −30 mV, following brief (20 ms) pulses to potentials ranging from −30 to +50 mV. Figure 4*A* shows the curves obtained by averaging the results obtained in euthyroid ($n = 7$) or hyperthyroid ($n = 8$) cells. No significant changes could be seen in the hyperthyroid cells. By fitting a Boltzmann equation to the data (continuous line), it was found that the half-inactivation membrane potentials ($V_{\frac{1}{2}}$) (uncorrected for junction potentials) were −4·2 and −7·8 mV for the euthyroid and hyperthyroid cells, respectively. The slope factors were −11·8 and −10·4 mV, respectively.

The steady-state inactivation parameter was measured by giving long (1·5 s) prepulses to potentials ranging from −80 to +15 mV, followed by a test pulse to +40 mV. Figure 4*B* shows the mean inactivation curve. Boltzmann curves were fitted to the mean values obtained in ten (normal) or eight (hyperthyroid) cells. For the steady-state inactivation parameter, $V_{\frac{1}{2}}$ values were −26·0 and −19·7 mV in normal and hyperthyroid cells, respectively. The slope factors were 10·7 and 8·4 mV, respectively. The 'real' half-activation and inactivation voltages are 10 mV more negative than indicated, due to the junction potential present in the electrode filling solution (see Methods). The membrane potential at which 50% inactivation occurs is close to that measured (in the same preparation) by Hiraoka & Kawano (1989). Their activation curve is slightly skewed (their Fig. 3) and less symmetrical with the inactivation curve, in comparison to ours. In our experiments, comparing euthyroid and hyperthyroid groups did not show any significant shift in the voltage dependence of the activation gating parameters, and a slight depolarizatory shift in the inactivation parameter. Such a shift cannot account for the enhancement of peak I_t at positive membrane potentials. However, it may have some importance at plateau potentials, since the shift in the voltage dependence of inactivation will increase the steady-state 'window' current. Superposition of the activation and inactivation curves, for euthyroid and hyperthyroid conditions, showed that a small increase indeed occurs in the 'window' current, as illustrated in Fig. 5. This can contribute

substantially to the termination of the action potential plateau, since at that time all currents are small. If, in addition, the maximal conductance of the channel (g_{max}) increases (see below), even small changes in the 'window' current can be important, since they will be amplified (the steady-state current is the product of the values of

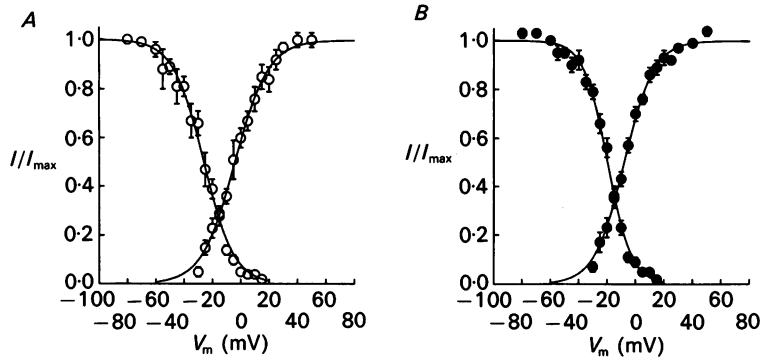

Fig. 5. Superimposed steady-state activation and inactivation gating parameter values, as a function of membrane potential, for euthyroid (*A*) and hyperthyroid (*B*) ventricular myocytes. The mean values (±S.E.M.) are plotted as a fraction of the maximal current I_{max}. The protocols used were those shown in Fig. 4. The overlapping regions show at what potentials there is a 'window' current, and what its amplitude will be (the product of the activation and inactivation values, g_{max}, and the driving force). The 'window' current is slightly larger in the hyperthyroid cells, and this is further amplified by the elevated g_{max} (see text).

the activation and inactivation parameters, g_{max} and the driving force). Thus, in hyperthyroid hearts, the shorter action potential duration may be due to a larger 'window' current of I_t.

In addition to examining possible changes in the voltage dependence of steady-state activation and inactivation, the following point was considered. The peak I_t in hyperthyroid myocytes might be larger if a faster activation and/or slower inactivation is present. We measured activation times by fitting exponential curves to the onset of I_t at +40 mV. At this potential there is very little interference from I_{Na} (in a small number of experiments the addition of TTX to block the sodium current did not change the measurements). In normal myocytes, the mean ($n = 6$) time constant of activation was 2·43 ± 0·19 ms. This is in very close agreement to values reported by Giles & van Ginnekken (1988) and by R. B. Clark, W. R. Giles & Y. Imaizumi (personal communication). In the hyperthyroid myocytes, the value was 1·79 ± 0·15 ms ($n = 8$).

The inactivation kinetics were more difficult to quantify, since the inactivation of the current had a multi-exponential decay time course, which varied considerably. A curve-fitting program was used to analyse inactivation (at +40 mV, 22 °C). Typically, two exponentials gave the best fits (see Clark *et al.* 1988). In normal myocytes, the fast time constant was 68·1 ± 13·1 ms (twelve cells), ranging from 21·7 to 155·2 ms. The slow time constant averaged 434·0 ± 97·5 ms, ranging from 146 to 1295 ms. In hyperthyroid myocytes, the fast component averaged 51·9 ± 7·9 ms (fifteen cells), ranging from 12·1 to 108·1 ms. The slow component averaged

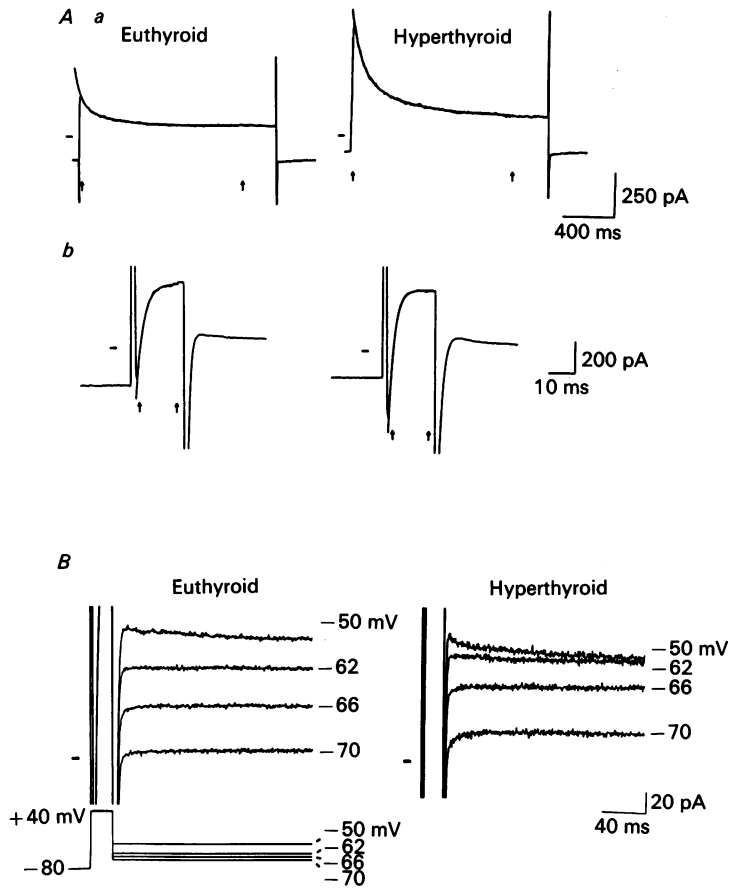

Fig. 6. *A*, the kinetics of onset and inactivation of I_t in euthyroid and hyperthyroid cells. The time course of inactivation was analysed using the DISCRETE program for multi-exponential curve fitting. The best fits were for bi-exponential decays, with the mean fast time constant being $68{\cdot}1 \pm 13{\cdot}1$ ms in euthyroid ($n = 12$) and $51{\cdot}9 \pm 7{\cdot}9$ ms ($n = 15$) in hyperthyroid cells. The mean slow time constants are 434·0 and 334·5 ms, respectively. Two typical examples are shown in *Aa*, with the current records superimposed by the best-fitting curves. The activation was best fitted with single exponentials, with the time constants averaging $2{\cdot}43 \pm 0{\cdot}19$ ms in euthyroid ($n = 6$) and $1{\cdot}79 \pm 0{\cdot}15$ ms in hyperthyroid cells ($n = 8$). Two examples are shown in *Ab*, with the currents (at +40 mV) and the best fits superimposed. The arrows denote the time windows used for the curve fitting. *B*, the reversal potential of I_t in euthyroid and hyperthyroid myocytes. A two-pulse protocol, with an activating pulse to +40 mV followed by test pulses to different potentials was used. The currents in response to the different test potentials can be seen to reverse at close to −66 mV in both groups. The true reversal is close to −76 mV (10 mV more negative due to the junction potential between the pipette and the bath – see Methods). The similar reversal potential implies unchanged selectivity of the channel in the hyperthyroid state.

$334{\cdot}5 \pm 75{\cdot}9$ ms, ranging from 43 to 1124 ms. Sample current traces showing superimposed best-fit curves for activation (on the left) and inactivation (on the right) are shown in Fig. 6*A*.

Thus, as can also be judged from Figs 2 and 3, inactivation was usually faster in

hyperthyroid myocytes, rather than slower. It is thus very unlikely that changes in the kinetics of onset and decay of I_t are important underlying mechanisms producing an augmentation in peak I_t.

Reversal potential

The selectivity of the channel was assessed by measuring the reversal potential for I_t in normal and hyperthyroid myocytes. Using a brief activating pulse, tails were elicited upon returning to different membrane potentials. The precise value of reversal is difficult to determine. However, it could be determined that any shift in reversal potential (E_{rev}) could not be larger than 5 mV. In both euthyroid ($n = 5$) and hyperthyroid ($n = 4$) myocytes, the measured reversal potential was close to -66 mV (-76 mV when corrected for junction potential – see Methods). This suggests that no major changes occur in the selectivity of the I_t channel. Sample traces of such an experiment are shown in Fig. 6*B*.

It thus seems that the increase in I_t amplitude arises from a larger I_t conductance. This could result from an increase in the unitary channel conductance and/or in the number of I_t channels in the cell membrane, as well as in mean channel open times. This issue was examined by recording single-channel activity.

Single-channel recordings

The first striking finding in hyperthyroid cells was the fact that I_t channel activity could be seen in most patches (nine out of twelve), in contrast to a success rate of 10–30% in normal myocytes (R. B. Clark & W. R. Giles, unpublished observations). Furthermore, in four of these patches, more than one channel was present. This is very suggestive of an increased number of channels in the membrane. The current–voltage (I–V) relationship was established for nine patches, in a voltage range of 50–125 mV positive to the resting membrane potential. The relationship was linear in this range. Sample current traces in response to different voltage steps in one patch are shown in Fig. 7 (left). On the right are the I–V relationships for all nine patches. The best-fitting linear regression line through all the data points had a slope of 12·0 pS, which is the average unitary conductance of the channel. This is very close to the slope conductance found in normal ventricular cells (Fedida & Giles, 1991). The (extrapolated) intercept on the voltage axis was 8·1 mV positive to rest.

Thus, since the unitary channel conductance is unchanged in the hyperthyroid myocytes, the larger macroscopic currents imply an increase in the number of channels in the membrane. One possible consideration is a change in the peak probability of I_t channel openings. However, at positive voltages, open probability (P_o) values of around 0·8 have been reported for normal myocytes (Fedida & Giles, 1991). Thus, an increase in probability cannot account for the large (4- to 5-fold) increase in macroscopic currents.

Atrial cells

The next stage was to examine atrial cells. This issue is of great importance, since in atrial tissue hyperthyroidism does not affect the myosin distribution. It was thus of importance to establish whether ionic currents are also resistant to changes under hyperthyroid conditions. I_t current densities were compared in two groups of atrial myocytes. Figure 8 (left) shows typical current traces obtained (at 22 °C, 0·2 Hz) in

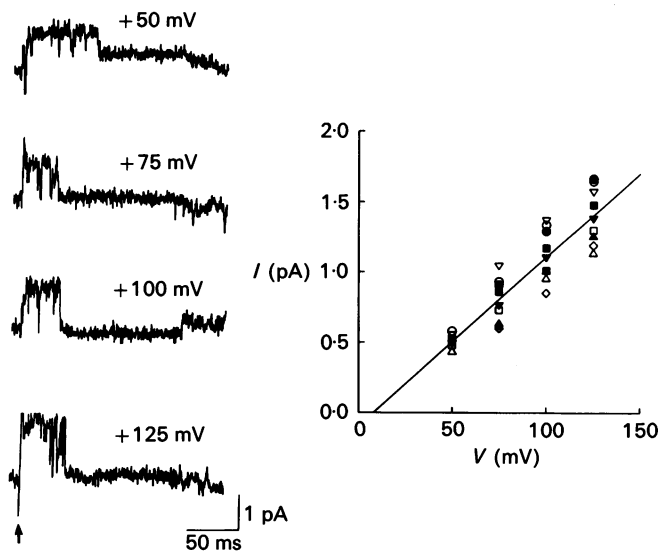

Fig. 7. Single I_t channel activity in a hyperthyroid ventricular myocyte at 22 °C. The sample current records shown on the left were obtained in response to depolarizing steps (150 ms in duration), given from −50 mV to the membrane potentials indicated (all values are with respect to the resting membrane potential). The rate of stimulation was 0·2 Hz. The arrow (bottom left) indicates the start of the depolarizing pulses. The currents were filtered −3 dB at 1 kHz. On the right are the current amplitudes for the different voltage steps, obtained in nine different patches. The regression line obtained by least-squares fitting is also shown. The slope is 12·0 pS, which is the mean unitary channel conductance.

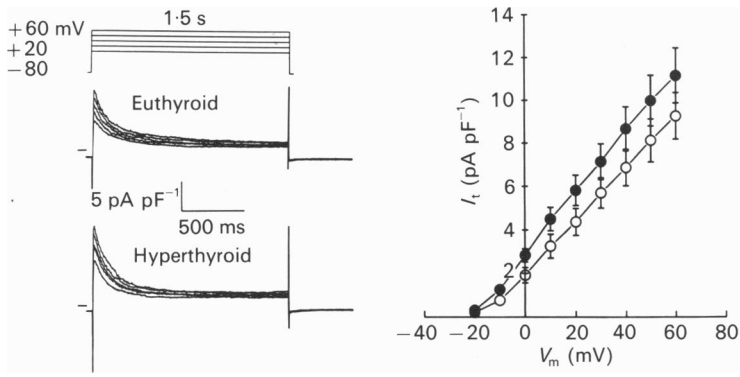

Fig. 8. Transient outward potassium currents in euthyroid and hyperthyroid atrial cells, at 22 °C. Superimposed current records from typical cells are shown on the left. The currents are elicited by 1·5 s depolarizations to potentials between +20 and +60 mV, at 0·2 Hz. $CdCl_2$ (0·5 mM) is used to block calcium currents. On the right are current–voltage relationships for the two groups, obtained by using mean (with standard error bars) current densities (pA pF^{-1}) for the different membrane potentials. ○, euthyroid cells and ●, hyperthyroid cells, showing only a small increase in the hyperthyroid atrial cells, in marked contrast to ventricular cells.

response to depolarizing steps from −80 mV. The examples are from a normal (euthyroid) myocyte, and from an atrial cell from a hyperthyroid rabbit. On the right are the mean values of current densities (for each cell the current amplitude was divided by the cell capacity) for the different membrane potentials. In striking

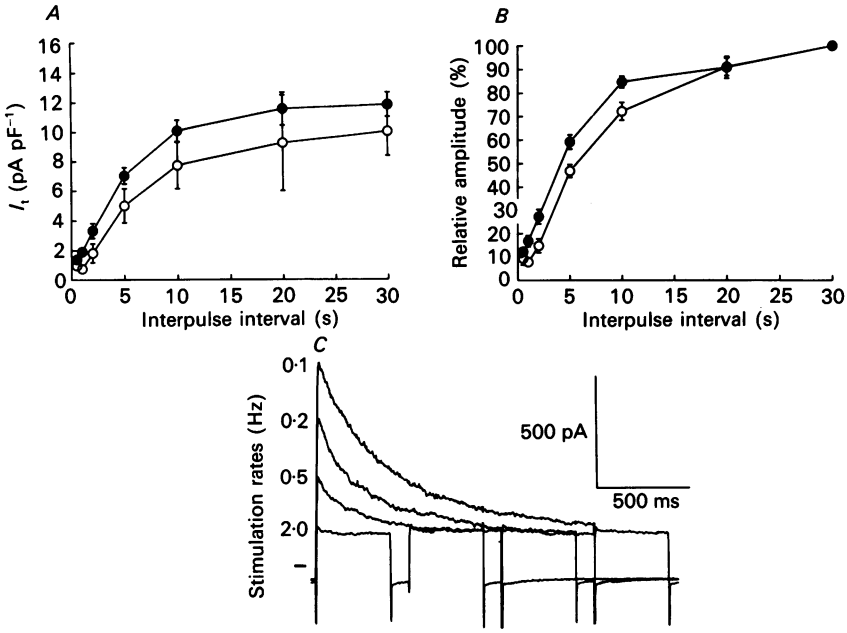

Fig. 9. The rate dependence of I_t in euthyroid (○) and hyperthyroid (●) atrial cells at 22 °C. *A*, mean I_t amplitudes measured during steady-state stimulation at the different interpulse intervals. A slight increase in amplitude is seen at all rates in the hyperthyroid cells. *B*, relative availability of the current at the different rates (100% defined as the amplitude at stimulation intervals of 30 s). A slight speeding up of reactivation in the hyperthyroid cells is evident, although at the higher rates (shortest intervals) there is not much difference between the two groups (see text). *C*, current records from hyperthyroid atrial cells at steady-state stimulation rates of 0·1, 0·2, 0·5 and 2 Hz. The high rates suppress I_t, as in normal myocytes. This is in contrast to hyperthyroid ventricular cells (Shimoni *et al.* 1992, Fig. 3).

contrast to the 4- to 5-fold increase seen in ventricular cells (Fig. 2), the increase in I_t in hyperthyroid atrial cells ($n = 21$) is minimal, in comparison to the normal current densities ($n = 13$). This is true for the entire range of membrane potentials studied. Calcium currents were blocked by 0·5 mM $CdCl_2$.

In the hyperthyroid ventricular cells, a change was found in the rate dependence of I_t. This was examined in the atrial cells as well. The effect of hyperthyroidism on the magnitude of I_t was studied (five cells) over a wide range of stimulation rates (0·033–2 Hz). The slight enhancement of I_t was obvious for the entire range, as shown in Fig. 9*A*. The relative current magnitude (normalized to the fully available current at the slowest stimulation rate) is also slightly larger at fast and intermediate stimulation rates, indicating a small enhancement of the speed of repriming of the current (Fig. 9*B*). However, this is insufficient to maintain I_t at high rates, in

contrast to the situation in hyperthyroid ventricular cells, where I_t persists at the higher rates. Thus, in atrial cells, the current is suppressed at a stimulation rate of 2 Hz. This can be seen in Fig. 9*C*, which shows superimposed current traces obtained at different rates (steady-state current traces are shown).

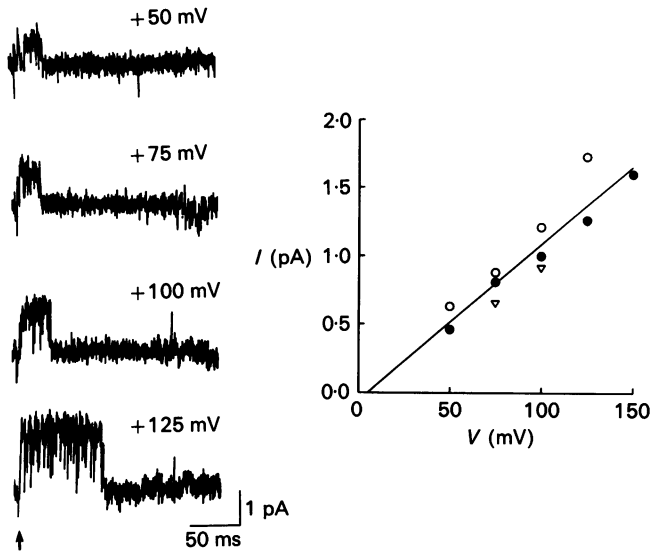

Fig. 10. Single-channel activity in a hyperthyroid atrial myocyte (at 22 °C). On the left are samples current traces (filtered −3 dB at 2 kHz) obtained in response to 150 ms depolarizing steps, given at a rate of 0·2 Hz, to the membrane potentials indicated. On the right are the current amplitudes in three patches, plotted against the membrane potentials. The holding potential was −50 mV (all values are with respect to the resting membrane potential). The best-fitting line, obtained by least-squares fitting, is also shown. The slope is 11·4 pS, which is the mean unitary channel conductance.

Thus, not only is the current amplitude unchanged in atrial cells from hyperthyroid animals, but a basic kinetic property is also similar to the normal, in contrast to the hyperthyroid ventricular cells.

To further extend the comparison with ventricular cells, single-channel activity was also recorded in another short series of experiments, using the cell-attached mode of recording. The incidence of finding active patches was 50% (three out of six trials), which is close to that in normal atrial myocytes (Clark *et al.* 1988). In no case was there more than one channel per patch, in contrast to ventricular myocytes from hyperthyroid rabbits. For each patch, different voltage steps were given, allowing the construction of current–voltage relationships. These were linear in the range 50–125 mV positive to the resting membrane potential. The slope conductance, obtained from the average values, was 11·4 pS, which is comparable to the normal value (Clark *et al.* 1988). The intercept with the voltage axis was at 4·9 mV positive to the resting potential. These results are illustrated in Fig. 10.

Finally, it was of interest to establish whether the altered rate dependence of the macroscopic current, seen in ventricular myocytes and not in atrial myocytes (from

hyperthyroid hearts), could also be observed in single-channel recordings. In the single-channel recordings as well, increasing the rate of depolarizations to 12 Hz did not abolish channel activity in ventricular myocytes, whereas activity in atrial cells ceased. This was true for the entire range of membrane potentials tested. This is

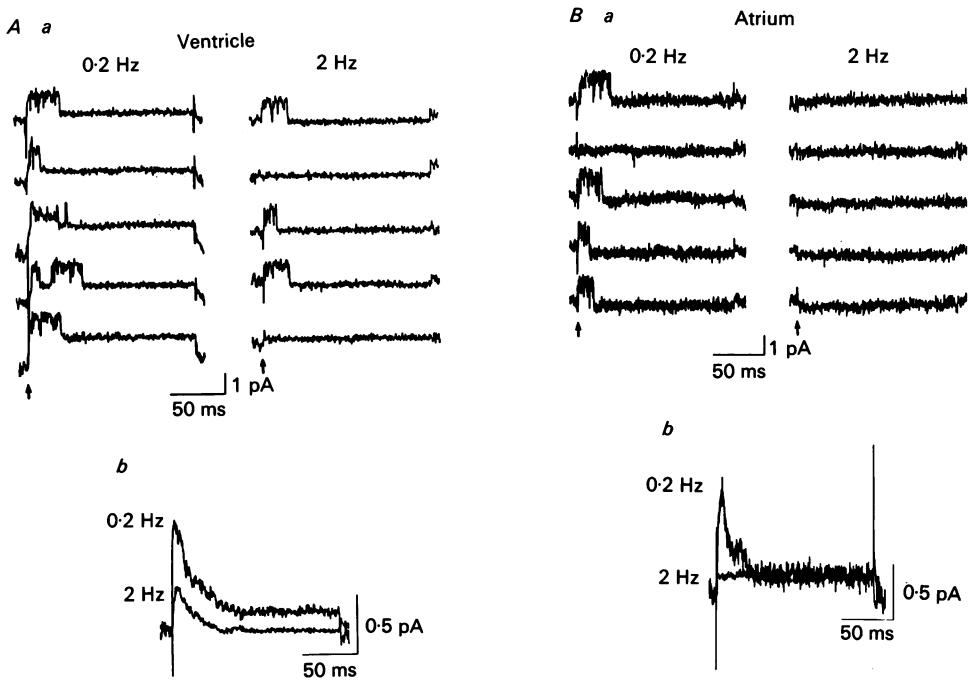

Fig. 11. Single-channel two activity in hyperthyroid ventricular and atrial myocytes at two rates of stimulation, at 22 °C. The current traces were obtained in response to steps from −50 to +100 mV, relative to rest. *Aa*, sample sweeps in a ventricular cell at 0·2 Hz (left), with sample traces at 2 Hz on the right (filtered at 1 kHz). *Ab*, ensemble averages (thirty sweeps) from the two stimulation rates are superimposed, showing a considerable amount of current still present (although reduced) at 2 Hz. *Ba*, sample current traces (filtered −3 dB at 2 kHz) from an atrial cell, at 0·2 Hz on the left, and at 2 Hz on the right. *Bb*, superimposed ensemble averages of thirty sweeps obtained at the two stimulation rates. In contrast to hyperthyroid ventricular cells, single-channel activity in hyperthyroid atrial cells is suppressed at 2 Hz.

illustrated in Fig. 11, which shows current traces obtained at 0·2 and 2 Hz, for a step to +100 mV with respect to rest, for ventricular cells (*A*) and atrial cells (*B*). *Ab* and *Bb* are superimposed ensemble averages for the two rates. These greatly resemble the whole-cell records. For the ventricular cells, peak open probabilities were calculated at 0·2 and 2 Hz, over a potential range of 50–125 mV positive to the resting potential. This was done by dividing the average ensemble magnitude (divided by two for patches with two channels) by the mean current amplitude at each potential. The results are shown in Fig. 12. Although the trend (increasing open probability at larger depolarizations) is clear, more data will be needed to determine the precise voltage dependence of the open probability.

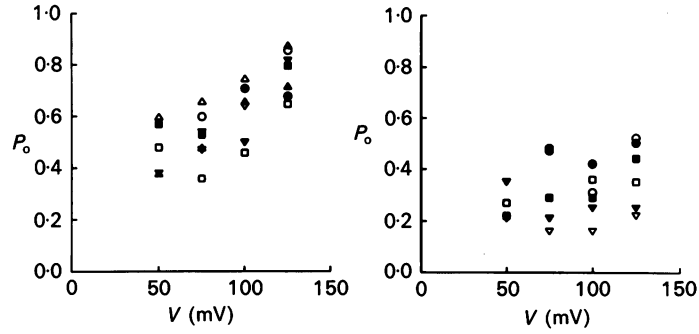

Fig. 12. Peak open probabilities (P_o) for I_t single channel activity in hyperthyroid ventricular cells. Probabilities were calculated by dividing the ensemble average amplitude by the mean current amplitude, for each membrane potential. In the case of two active channels in the patch, the ensemble magnitude was first divided by two. On the left are the results from six patches, showing a range of (approximately) 0·4–0·8 for peak open probabilities, between 50 and 125 mV positive to the resting membrane potential, at a stimulation rate of 0·2 Hz. On the right are the probabilities at 2 Hz, ranging (approximately) from 0·2 to 0·4. The voltage dependence may be weaker at 2 Hz, but more data is required to establish this.

DISCUSSION

The results presented here are the first detailed study of the transient outward current recorded from single myocytes obtained from hyperthyroid mammalian hearts. The most prominent feature found is a significant enhancement of the macroscopic I_t current in ventricular, but not in atrial myocytes (Figs 2 and 8).

Although the results presented here were obtained at 22 °C, similar results were obtained at 32 °C (not shown). The prominence of I_t and its physiological relevance in hyperthyroid conditions can also be inferred from the fact that the complex action potential configuration seen at 22 °C (Fig. 1) was also recorded in the warm (32–34 °C). This was also the case for multicellular recordings, as found by Sharp *et al.* (1985), who suggested an augmented I_t as the underlying mechanism.

The single-channel recordings showed that the unitary conductance was unchanged, and thus that the larger current density must mean a larger channel density. The cell membrane thus contains a larger number of channels in the hyperthyroid state. Since elevated thyroxine levels affect the transcription of a variety of genes (Samuels *et al.* 1988), it is most likely that the rate of synthesis of I_t channel proteins is augmented.

However, the effects of elevated thyroxine levels on gene transcription appear to have been selective, with no effects on atrial cells. This resembles the selectivity with regard to the expression of the genes encoding for myosin heavy chains (Banerjee, 1983; Nadal-Ginard & Mahdavi, 1989). Thus, there seems to be a more generalized pattern of response, in which ventricular cells respond in a variety of ways, whereas atrial cells do not respond at all. This implies that if demands or circumstances

change, cells can respond by adapting both electrical and mechanical characteristics, but that not all cells will necessarily do so. The reasons for this selectivity are not clear, but may be related to the changes in energetic efficiency associated with myosin isoform changes (Kissling, Rupp & Jacob, 1987). The adaptation of atrial and ventricular cells may differ because of differences in energetic efficiency of their action under different metabolic conditions.

Several possible mechanisms could determine the selective action of thyroxine. On one hand, there may be a very fundamental (unknown as yet) regulatory process which determines the density of channels in the membrane. In ventricular cells, the baseline density is relatively low, with current densities that are 3- to 7-fold lower than those in atrial cells (at comparable membrane potentials, rates of stimulation and temperatures). It is therefore possible that the regulating mechanism is permissive to the synthesis and/or insertion of additional channels into the membrane. In the atrial cells, the channel density may be close to saturation, so that these cells 'resist' the addition of further channels into their membranes.

The results also show that in addition to the magnitude change, there was also a selective change in the rate dependence of I_t. Ventricular cells show a faster repriming, or reactivation of the current (Fig. 3) whereas atrial cells do not (Fig. 9). This explains the effects shown in Fig. 1*C*, whereby 4-AP prolongs the action potential even at high stimulation rates, since there is still substantial I_t to be suppressed. This selective change is strikingly apparent at the single-channel level as well, as shown in Fig. 11. There are several functional implications for these changes in I_t. First, as mentioned above, the hyperthyroid heart is characterized by tachycardia, in which the action potentials must be shorter than normal. A larger outward I_t current (which persists at high rates in contrast to the situation in normal heart) can facilitate action potential abbreviation. This would occur by an early repolarization which would reduce the plateau level to more negative membrane potentials. This, in turn, will directly reduce the influx of calcium, as well as slightly increase I_{K1} (the inwardly rectifying potassium current) which will then determine the timing of the final repolarization (Shimoni, Clark & Giles, 1992). A potentially valuable advantage of more negative plateau levels is the enhancement of calcium removal by the voltage-dependent sodium–calcium exchanger (Barcenas-Ruiz & Wier, 1987). Thus, some protection against calcium overload at the high heart rates can also be achieved. In atrial cells the plateau is much less prominent (Giles & Imaizumi, 1988), so that the changes in action potential duration are perhaps less important.

An additional implication of an abbreviated action potential may be the disposition to ventricular arrhythmias, which often accompany hyperthyroidism (Symons, 1979).

In addition to an augmented conductance, other mechanisms could lead to larger macroscopic I_t currents. Several of these were examined and ruled out. Thus, it was found that the kinetics of activation and inactivation, and the voltage dependence of these parameters were practically unchanged (Figs 4–6). However, the 'window' current which arises from the overlap between the voltage dependence of activation and inactivation is affected. The augmented conductance (g_{max}) entails a larger 'window' current in itself (see text). In addition the slight shift in the inactivation

voltage dependence also contributes to a larger 'window' current. This window current, which may play a very crucial role in repolarization, can contribute to the abbreviation of the action potential in hyperthyroid conditions.

In summary, more active channels are present in hyperthyroid ventricular cells. These channels have a faster repriming, and are thus also changed in some aspects of their regulation. It is unclear if there is a formation of new channels which possess new characteristics or whether there are independent changes in channel properties or in channel regulation. Some of these issues are presently under investigation.

A final consideration is the existence of a calcium-activated (potassium or chloride) outward current in these cells (Hiraoka & Kawano, 1989; Zygmunt & Gibbons, 1991). This current could not be recorded in our experiments, since $CdCl_2$ was used to block calcium currents. One may speculate that if a shortening of action potential duration is achieved at high rates by an augmentation of outward currents, it would be advantageous if I_t were augmented, rather than the calcium-activated outward current, since augmenting the latter would necessitate increasing the calcium transient. We cannot rule out that the calcium-activated current is also increased in the hyperthyroid state, although a major role is unlikely since the magnitude of this current is normally 10- to 12-fold smaller than I_t, at plateau potentials (Giles & Imaizumi, 1988).

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