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# Hypothyroidism Decreases the ATP Sensitivity of $K_{ATP}$ Channels from Rat Heart

**P. Light[1,2], Y. Shimoni[1], S. Harbison[1], W. Giles[1], R.J. French[1]**

[1]Department of Physiology and Biophysics, University of Calgary, Alberta, Canada T2N 4N1

[2]Department of Pharmacology and Therapeutics, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1

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**Abstract.** The effects of thyroid status on the properties of ATP-sensitive potassium channels were investigated. Single-channel recordings were made using excised inside-out membrane patches from enzymatically dissociated ventricular myocytes from hearts of control and thyroidectomized rats and each group was studied with and without administration of thyroid hormone.

In patches excised from hypothyroid myocytes the $IC_{50}$ for ATP inhibition of $K_{ATP}$ channels was 110 μM. This value was 3-fold higher than the $IC_{50}$ in control myocytes (43 μM). Treatment of hypothyroid rats to restore physiological levels of thyroid hormone (tri-iodothyronine, $T_3$), resulted in a return to normal ATP-sensitivity ($IC_{50}$ = 46 μM). In patches from animals rendered hyperthyroid, the $IC_{50}$ for ATP was 50 μM and this value was not significantly different from the control. There was no difference in the cooperativity of ATP-binding (Hill coefficient, $n_H$) among control ($n_H$ = 2.2), hypothyroid ($n_H$ = 2.1), $T_3$-treated ($n_H$ = 2.0) and hyperthyroid groups ($n_H$ = 2.4). The unitary conductance was unchanged and there was no apparent change in intraburst kinetics between examples of single $K_{ATP}$ channels from control and hypothyroid rats. Action potentials recorded in myocytes from hypothyroid rats were significantly shortened by 50 μM levcromakalim, a $K_{ATP}$ channel opener ($P < 0.001$) but unchanged in control myocytes.

We conclude that hypothyroidism significantly decreased the ATP-sensitivity of $K_{ATP}$ channels, whereas the induction of hyperthyroid conditions did not alter the ATP-sensitivity of these channels. Thus, hypothyroidism is likely to have important physiological consequences under circumstances in which $K_{ATP}$ channels are activated, such as during ischemia.

*Correspondence to:* P. Light

**Key words:** ATP-sensitive potassium channel — Ventricular myocytes — Hypothyroidism — Patch clamp

## Introduction

A direct link between the metabolic state of cardiac cells and their electrical activity can occur through the activation of ATP-dependent potassium current, $I_{K(ATP)}$ (Noma, 1983). This current has been extensively studied (for reviews *see* Nichols & Lederer, 1991; Wilde & Janse, 1994), and the pore-forming subunit and associated sulfonylurea receptor have recently been cloned (Inagaki et al., 1995,1996). Some of the mechanisms for regulation of this channel have been elucidated. For example, the channel is known to be closed by increasing ATP levels and to be modulated also by intracellular $Mg^{2+}$, ADP and pH (Nichols & Lederer, 1991; Fan & Makielski, 1993). $K_{ATP}$ channels can be activated pharmacologically by $K^+$ channel ''openers'' such as pinacidil and levcromakalim (Sanguinetti et al., 1988; Nakayama et al., 1990), and inhibited by sulfonylurea compounds such as glibenclamide. Recently, a significant modulation by a G protein (Ito et al., 1994) and a regulatory role for protein kinase C have also been described (Hu et al., 1996; Light et al., 1996). Taken together, these findings suggest that $I_{KATP}$ may be involved in cardio-protection during ischemic episodes or in ischemic preconditioning (Liu et al., 1996).

Very few studies have addressed the regulation of $I_{K(ATP)}$ under conditions where cellular metabolism is altered for extended time periods as may occur during hormonal imbalance. A key determinant of cellular metabolism is the level of thyroid hormone (Dillman, 1990). Changes in the levels of tri-iodothyronine ($T_3$) are known to significantly alter oxygen consumption as well as glycolytic rate (Seymour, Eldar & Radda, 1990; Morkin, Flink & Goldman, 1996). Over the last few years it has

become apparent that $T_3$ regulates several $K^+$ currents (Shimoni, Severson & Giles, 1995). It has been suggested that $I_{K(ATP)}$ may be preferentially dependent on glycolytically derived ATP (Weiss & Lamp, 1989), although a recent study suggests ATP derived from oxidative phosphorylation is more important (Shigematsu & Arita, 1997). Reduced $T_3$ levels may also affect glycolytic function (Gualberto, Molinero & Sobrino, 1987; Morkin et al., 1996). We therefore considered it to be of interest and importance to establish whether there are changes in $I_{K(ATP)}$ during the chronically depressed metabolic state achieved under the hypothyroid condition. Thus, we set out to determine whether thyroidectomy elicits any changes in the properties of $I_{K(ATP)}$, such as the current-voltage relationship, channel kinetics and ATP-dependence. Our results show that reproducible changes occur during the hypothyroid state, and that these changes can be reversed with $T_3$ treatment.

## Materials and Methods

### Experimental Groups and Cell Isolation

Single rat ventricular myocytes were prepared by enzymatic dispersion (*see* Shimoni et al., 1995 for details) from 4 groups of age-matched male Sprague-Dawley rats (i) control, untreated rats; (ii) thyroidectomized rats, used 4–6 weeks after surgery; (iii) thyroidectomized rats which received physiological doses of $T_3$ (10 μg/Kg, given daily for 5–6 days, 4 weeks after thyroidectomy); (iv) control rats receiving $T_3$ (250 μg/Kg, given daily for 5–6 days) to render them hyperthyroid. Measurement of $T_3$ levels from rats used in this study were pooled with values obtained previously in our laboratory using the same protocols and were as follows: Hypothyroid rats ($n = 12$) all had plasma $T_3$ levels of 0.4 nM or less (limit of detection). The thyroidectomized rats which received $T_3$ replacement 0.70 ± 0.14 nM ($n = 6$). $T_3$ concentrations in control rats were 0.78 ± 0.17 nM ($n = 18$). In control rats rendered hyperthyroid the $T_3$ levels were 3.37 ± 0.86 ($n = 6$).

### Solutions

The standard pipette solution used for excised, inside-out, patch recordings contained the following (in mM): NaCl 140; KCl 5; HEPES 10; $CaCl_2$ 1; $MgCl_2$ 1; glucose 10 at pH 7.4. The standard bath solution contained (in mM): K Aspartate 130; KCl 10; HEPES 10; EGTA 1; $MgCl_2$ 1.4; glucose 10. The pH was adjusted to 7.4 with KOH.

### Action Potential Recordings

Action potentials were recorded (under current clamp) from single right ventricular myocytes from control and hypothyroid rats, using the conventional whole cell, suction electrode recording method. The bath solution contained (in mM): 140 NaCl; 5.4 KCl, 1.0 $CaCl_2$; 1.0 $MgCl_2$; 5 HEPES; 5.5 glucose (pH 7.4). The pipette solution contained: 130 K-aspartate; 10 KCl; 1.4 $MgCl_2$; 10 HEPES; 10 glucose, 1 MgATP, at a pH of 7.4. All recordings were done at room temperature (20–22°C).

### Single-channel Recordings

Standard patch-clamp techniques (Hamill et al., 1981) were used to record single-channel currents in the inside-out patch configuration. The internal faces of the patches were directly exposed to test solutions via a multi-input perfusion pipette. Single-channel currents were recorded at fixed holding potentials, amplified (Axopatch 200, Axon Instruments, Foster City, CA), digitized (Neuro-corder DR-384, Neuro Data Instruments, New York, NY), and then stored on videotape. All data were sampled at 500 Hz and filtered at 200 Hz unless otherwise stated.

$K_{ATP}$ channel open probability was expressed as $NP_o$, the product of $N$, the number of channels in the patch, and $P_o$, the mean open probability. $NP_o$ was calculated by dividing the mean patch current (over a 10–30 sec test period) by the mean unitary current amplitude. For measurements of ATP sensitivity, $NP_o$ was usually expressed in normalized form for each patch, i.e., $NP_o$(test[ATP])/$NP_o$(zero ATP). Upon excision, patches were continuously exposed to 1 mM ATP, except for a brief exposure to 0 ATP at the beginning and end of experiments to estimate (i) the number of channels in a patch, and (ii) the degree of rundown (Kakei & Noma, 1984). Data from patches exhibiting >25% rundown were discarded.

### Unitary Conductances

Single-channel conductances were measured under symmetrical conditions using the standard internal solution as pipette solution. Mean unitary current amplitudes were calculated from the difference between peaks in a multiple Gaussian fit to all-points histograms from data segments of 10–30 sec in duration. Mean open and closed dwell times were generated from events lists (2818–10150 events) obtained from data segments 5–10 sec in duration.

### Statistics

Statistical significance was evaluated by Student's paired *t* test. Differences with values of probability ($P < 0.05$) were considered to be significant. All values in the text are mean ± SEM.

### Drugs and Reagents

ATP (as MgATP, Sigma Chemical, St. Louis, MO) was added as required from a 10-mM stock, which was prepared, immediately before use. Glibenclamide (Sigma) was stored as a 10-mM stock solution in dimethyl sulfoxide (DMSO). Levcromakalim was generously provided by SmithKline Beecham Pharmaceuticals (UK) and stored as a 50-mM stock in DMSO. Both glibenclamide and levcromakalim were diluted to the required concentration immediately before use. The concentration of DMSO used in this study (<0.1%) has previously been shown to be without effect on $K_{ATP}$ channels (Findlay, 1992).

## Results

### Thyroid Status and ATP-Sensitivity

Spontaneous $K_{ATP}$ channel activity was inhibited by ATP in a concentration-dependent manner in both control and hypothyroid membrane patches. However, the $IC_{50}$ for ATP inhibition of $K_{ATP}$ channels was increased

from 43 μM in control to 110 μM in hypothyroid myocytes (*see* Fig. 1*A* and *B*, $P < 0.05$). In $T_3$-treated hypothyroid animals, ATP sensitivity reverted to control levels ($IC_{50}$ = 46 μM). In hyperthyroid animals, the $IC_{50}$ for ATP-inhibition was not significantly different from controls ($IC_{50}$ = 50 μM). The ATP concentration-response curves in Fig. 2*B* were fitted using the least squares method (*see* equation below) to give values for the Hill coefficient ($n_H$) of ATP-binding of 2.1 (control), 2.2 (hypothyroid), 2.0 ($T_3$-treated) and 2.4 (hyperthyroid).

$$\text{Normalized } NP_o = NP_o/NP_{o(\max)} = 1/[1 + ([ATP]/IC_{50})^{n_H}].$$

Here, $NP_o$ is the product of $P_o$ (open probability) and $N$ (the number of channels in a patch). $NP_{o(\max)}$ denotes $NP_o$ in the absence of ATP. [ATP] is the test ATP concentration, $IC_{50}$ signifies the concentration of ATP at which half-maximal inhibition occurs.

### Single-channel Conductance and Channel Density

Under symmetric conditions (140 mM $K^+$) single $K_{ATP}$ channels from control myocytes displayed a unitary slope conductance, at negative potentials, of 66 ± 3.2 pS ($n$ = 3 patches, *see* Fig. 2). Single channels from hypothyroid myocytes exhibited a similar unitary slope conductance to control (66 ± 4.2 pS, $n$ = 3 patches). Channels from hypothyroid myocytes displayed somewhat less inward rectification at positive potentials when compared to control; at a holding potential of +60 mV the mean unitary currents from hypothyroid and control channels were 2.07 ± 0.31 and 1.45 ± 0.14 pA respectively ($P < 0.05$ *see* Fig. 2).

To assess whether thyroid status affects channel density, we estimated a lower limit on the number of channels in each patch using pipettes of a similar resistance and tip diameter. A comparison of the number of channels in excised patches, shows no differences in $K_{ATP}$ channel density between control (19.4 ± 3.8 channels, $n$ = 14 patches), hypothyroid (20.2 ± 5.5 channels, $n$ = 12 patches), hypothyroid $T_3$-treated (21.2 ± 5.0 channels, $n$ = 6 patches) and hyperthyroid (17.6 ± 3.4 channels, $n$ = 5 patches) conditions. None of these values were significantly different from each other. Channel density was calculated by dividing the mean current under maximally activating conditions (0 ATP) by the single channel current amplitude. 10–20-sec long segments of data were used in these analyses. Patches exhibiting $K_{ATP}$ channel rundown were not included (see Materials and Methods).

### Kinetic Analysis

The high density of $K_{ATP}$ channels in cardiac tissue makes single channels patches highly improbable. However, it was possible to analyze the single-channel open and closed times within a burst of activity from single channels, one from a control myocyte and one from an hypothyroid myocyte, in the absence and presence of internal ATP. We were unable to obtain patches containing only a single channel from the $T_3$-treated and hyperthyroid groups. The mean open times for control and hypothyroid myocytes were 4.01 and 4.38 msec respectively. The closed time was identical for control and hypothyroid myocytes (0.43 msec, *see* Fig. 3). Both the open and closed time distributions were well fitted by single exponentials for either control or hypothyroid channels. To investigate whether the intraburst kinetics were altered in the presence of internal ATP, we subjected the same two patches to 50 μM internal ATP. Under these conditions, the mean open and closed times for the control channel were 4.2 and 0.45 msec respectively. The mean open and closed times for the hypothyroid channel were 4.15 and 0.42 msec respectively. In addition, in the 6 patches containing a small number of channels (2–4) used to measure unitary conductance, there

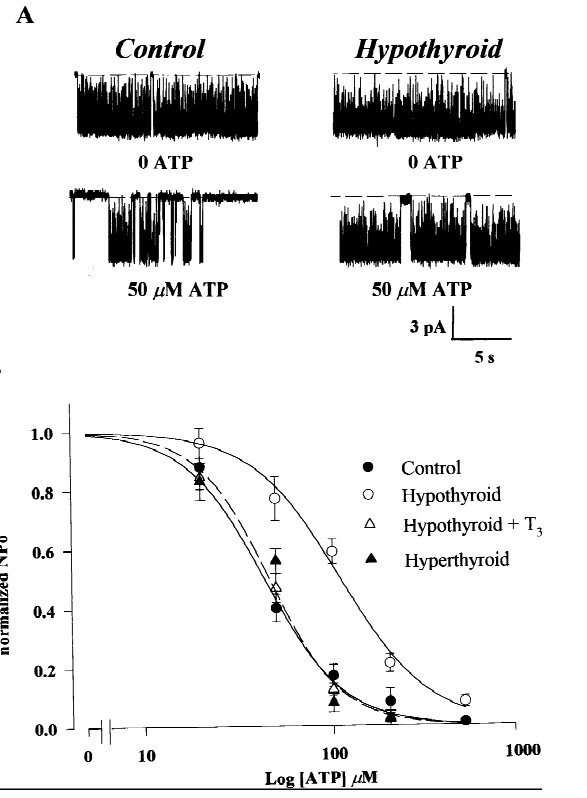


**Fig. 1.** (*A*) Single-channel recordings of $K_{ATP}$ channels from rat ventricle in symmetrical $K^+$ (140 mM) at a holding potential of −50 mV. $K_{ATP}$ channel activity, from control and hypothyroid myocytes, is shown at two different internal ATP concentrations, zero ATP and 50 μM. (*B*) Effect of ATP on the normalized open probability ($NP_o$) of $K_{ATP}$ channels from (●) control ($n$ = 14 patches), (○) hypothyroid ($n$ = 12 patches), (△) $T_3$ replacement ($n$ = 6 patches) and (▲) hyperthyroid ($n$ = 5 patches) groups.

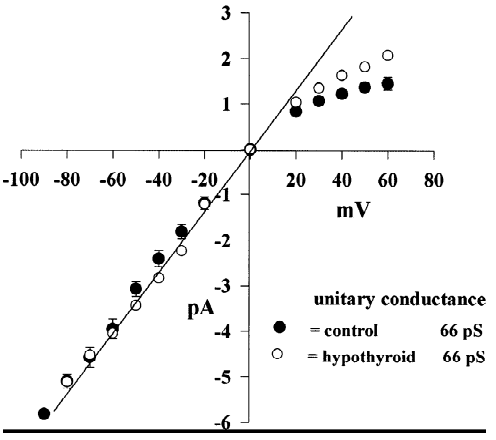


**Fig. 2.** Current-voltage relationships for single $K_{ATP}$ channels from (●) control and (○) hypothyroid rat ventricular myocytes. Recordings were made using symmetrical $K^+$ (140 mM). The unitary slope conductance at negative potentials was 66 ± 3.6 pS ($n = 3$) and 66 ± 4.2 pS ($n = 3$) for $K_{ATP}$ channels from control and hypothyroid myocytes respectively.

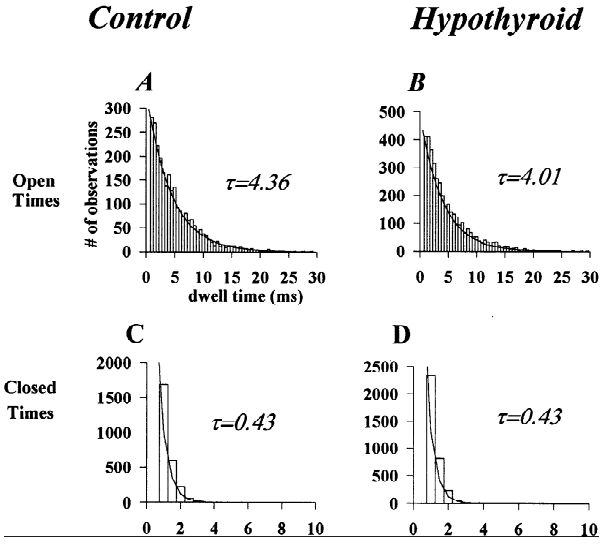


**Fig. 3.** Open and closed time distributions of single $K_{ATP}$ channels from control and hypothyroid groups. Open (*A* and *B*) and closed (*C* and *D*) time histograms were constructed from 10–15 sec of data from inside-out patches containing single $K_{ATP}$ channels. $K^+$ concentration was symmetrical (140 mM) and the holding potential was −50 mV. All histograms were fitted with a single exponential using a least-squares method. The time constants (τ) are shown above each curve. The sampling rate and filter frequency (−3dB) were 2 and 1 kHz respectively.

were no observable qualitative changes that suggested any differences in kinetics. Thus, intraburst kinetics appear to be independent of $[ATP]_i$ and the hypothyroid state.

### Levcromakalim Shortens the Action Potential in Hypothyroid Myocytes

The $K_{ATP}$ channel opener, levcromakalim, activates the channels, in part, by inducing a rightward shift in the ATP dose-response curve (Terzic, Jahangir & Kurachi, 1995). If this effect were additive with the similar change associated with reduced thyroid activity, one would expect electrical activity to be more sensitive to levcromakalim in hypothyroid myocytes than in controls, because the channels in the hypothyroid myocytes would be operating on a slightly steeper part of the ATP-dose response curve (*see* Fig. 1*B*). Thus, in a final series of experiments, we decided to test whether there were differential effects of levcromakalim (50 μM) on the action potential waveform in hypothyroid and control myocytes. As previously documented, the action potential is prolonged under hypothyroid conditions (Sharp et al., 1985). With 1 mM ATP in the pipette, 50 μM levcromakalim induced no significant change in $APD_{90}$ in control myocytes ($P > 0.05$, $n = 12$). However, in myocytes from hypothyroid rats, application of the same concentration of levcromakalim resulted in a significant reduction in $APD_{90}$ (−31 ± 8.7 msec, $P = 0.02$, $n = 6$) from the pretreatment $APD_{90}$ values (Fig. 4). This effect was reversed on levcromakalim washout, and was blocked by the addition of a $K_{ATP}$ channel inhibitor, glibenclamide (10 μM). Glibenclamide was without effect on control action potentials when applied alone.

## Discussion

The present results demonstrate that the $K_{ATP}$ channel, in addition to being regulated by a variety of pathways on a moment-to-moment basis, can also be altered by long-term hormonal regulation. In adult rat ventricle, plasma $T_3$ levels have clear effects on the ATP-sensitivity of the $K_{ATP}$ channels. Reduced plasma $T_3$ levels were associated with a significant increase in the $IC_{50}$ value for inhibition of the channel by ATP (Fig. 1*B*) of approximately 3-fold. Other single-channel parameters, such as unitary conductance, intraburst dwell times and channel density, were not affected by the thyroidectomy (Figs. 2, 3). By using $T_3$ replacement in thyroidectomized animals, it was established that the observed changes were a direct result of the fall in plasma $T_3$ levels.

These results, which agree well with findings in vascular smooth muscle from hypothyroid rats (Jagadish et al., 1996), may have significant implications for cardiac activity when thyroid activity changes. Under hypothyroid conditions, there is a well-documented attenuation of several cardiac $K^+$ currents (Shimoni et al., 1995), leading to prolongation of the action potential (Binah et al., 1987). The increase in the $IC_{50}$ of ATP inhibition of $K_{ATP}$ channels (Fig. 1), suggests that these channels are more likely to be open in hypothyroid animals. This enhanced $K^+$ current may play a significant role under ''normoxic'' hypothyroid conditions, by counteracting

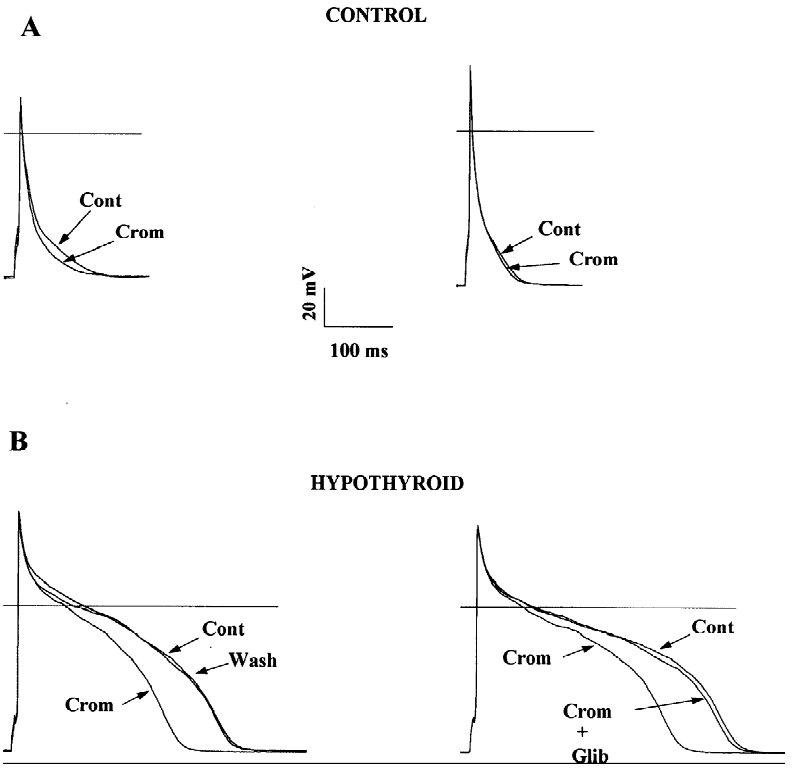


**Fig. 4.** Effects of levcromakalim on action potentials in single myocytes from normal (*A*) and hypothyroid (*B*) rats. Action potentials were elicited by steady stimulation (at 0.5 Hz) under control conditions (cont) and following addition of 50 μM levcromakalim (crom). With 1 mM ATP in the pipette solution, levcromakalim produced only a minimal shortening of action potential duration in normal cells (two examples shown in *A*). In marked contrast, there was a much greater shortening in the myocytes from hypothyroid rats (*B*). This effect could be reversed by washout (left panel), or, after a second exposure to levcromakalim in the same cell, by addition of 10 μM glibenclamide (Glib, right panel). The solid line indicates zero potential.

the reduction in other repolarizing $K^+$ currents. However, in hypothyroid animals, another functional role may become apparent during ischemia/hypoxia, when $K_{ATP}$ channels will be activated by a smaller reduction in intracellular ATP levels, as compared to euthyroid hearts under similar metabolic deprivation. It has been suggested that activation of cardiac $K_{ATP}$ channels can provide protection against ischemic damage (e.g., Nichols & Lederer, 1991), perhaps by shortening the action potential and reducing the accompanying contraction, thus conserving energy. Consistent with these results, Abe et al. (1992) have reported improved mechanical recovery following ischemic episodes in hypothyroid conditions. Due to the high input resistance of myocytes it has been proposed that only small changes in the open probability of $K_{ATP}$ channels can significantly alter the shape of the action potential (Nichols & Lederer, 1991). The change in ATP-sensitivity we observed is likely to play an important role in shaping the hypothyroid action potential under conditions in which $K_{ATP}$ channels are activated.

The shortening of the ventricular action potential induced by the $K_{ATP}$ channel opener, levcromakalim, in hypothyroid, but not control myocytes (Fig. 4), demonstrates the possibility of a stronger influence on the action potential waveform of $K_{ATP}$ channels under hypothyroid conditions. This may result from additive effects of the levcromakalim and the long-term modulation of the channel's ATP-sensitivity as thyroid activity changes. In addition, $K_{ATP}$ channels may play a more important role because of downregulation of other $K^+$ channels (Shimoni et al., 1995), which would result in a higher input impedance during the prominent plateau phase of the action potential observed in hypothyroid myocytes (Binah et al., 1987). Although the effects of levcromakalim on the AP duration are consistent with the reduced sensitivity to ATP, it should be noted that levcromakalim has inhibitory effects on other repolarizing $K^+$ currents, such as IKr and IKs in cardiac myocytes from guinea-pig (Heath & Terrar, 1994). If this effect occurs in rat myocytes, the amount of cromakalim-induced AP shortening would be less due to the reduction in these other repolarizing currents, however we still observed significant AP shortening under hypothyroid conditions.

The concentration of glibenclamide used in this study, 10 μM, is at the higher end of the range (1–10 μM) normally used to inhibit whole-cell and single channel cardiac $K_{ATP}$ currents in many studies. In addition, there are reports showing cardiac $K_{ATP}$ channel inhibition at nanomolar concentrations (Arena & Kass, 1989; Findlay, 1992). Higher concentrations than 10 μM were not used as they may lead to undesirable nonspecific effects such as chloride channel inhibition. Nevertheless, some possibility remains that 10 μM glibenclamide used in this study may not block 100% of channels. Because our experiments were performed at room temperature, care

should be taken in extrapolating to physiological conditions. Our conclusions are therefore based on the premise that there are no alterations in the long-term hormonal modulation effects on $K_{ATP}$ channels in the short time period during which recordings were made at a lower temperature.

The underlying mechanism for the change in ATP-sensitivity following thyroidectomy is not known. However, several lines of evidence provide some clues. It is known that thyroid hormones act through nuclear receptors, thereby regulating the expression of proteins (Polikar et al., 1993). Glucocorticoid hormones also affect the expression of voltage-gated $K^+$ channels in the heart (Levitan & Takimoto, 1994). It is, therefore, possible that expression of functionally different isoforms of $K_{ATP}$ channel subunits may underlie the changes in ATP-sensitivity which we observed. We have recently shown that protein kinase C (PKC) can activate $K_{ATP}$ channels in rabbit heart (Light et al., 1996). In addition, certain PKC isoforms are upregulated under hypothyroid conditions (Rybin & Steinberg, 1996). Thus, a maintained (tonic) enhancement of activity of PKC isoforms may be responsible for the differences in ATP sensitivity in our study. In our previous study, PKC elicited its effects on $K_{ATP}$ channel activity, in rabbit ventricular myocytes, via changes in the Hill coefficient of ATP-binding (Light et al., 1996). However, we observed no changes in the Hill coefficient under hypothyroid conditions. This suggests that if PKC is involved in the regulation of $K_{ATP}$ channel activity during hypothyroid conditions, then the mechanism of action is different from that previously observed.

In conclusion, we have demonstrated that $K_{ATP}$ channels are under the influence of long-term regulation by thyroid hormone. Given the complexity of $K_{ATP}$ channel regulation by a variety of factors (Noma, 1983; Nichols & Lederer, 1991; Light et al., 1996; Liu et al., 1996), extensive studies will be required to fully elucidate the exact pathway(s) by which $K_{ATP}$ channel activity is regulated as thyroid hormone levels change.

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