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Beta-Adrenergic Modulation of K^+ Current in Human T Lymphocytes

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Summary. The whole-cell voltage-clamp technique was employed to study the β-adrenergic modulation of voltage-gated K^+ currents in $CD8^+$ human peripheral blood lymphocytes. The β-receptor agonist, isoproterenol, decreased the peak current amplitude and increased the rate of inactivation of the delayed rectifier K^+ current. In addition, isoproterenol decreased the voltage dependence of steady-state inactivation and shifted the steady-state inactivation curve to the left. Isoproterenol, on the other hand, had no significant effect on the steady-state parameters of current activation. The isoproterenol-induced decrease in peak current amplitude was inhibited by the β-blocker propranolol. Bath application of dibutyryl cAMP (1 mM) mimicked the effects of isoproterenol on both K^+ current amplitude and time course of inactivation. Furthermore, the reduction in the peak current amplitude in response to isoproterenol was attenuated when $PKI_{5\text{-}24}$ (2–5 μM), a synthetic peptide inhibitor of cAMP-dependent protein kinase, was present in the pipette solution. The increase in the rate of inactivation of the K^+ currents in response to isoproterenol was mimicked by the internal application of GTP-γ-S (300 μM) and by exposure of the cell to cholera toxin (1 μg/ml), suggesting the involvement of a G protein. These results demonstrate that the voltage-dependent K^+ conductance in T lymphocytes can be modulated by β-adrenergic stimulation. The effects of β-agonists, i.e., isoproterenol, appear to be receptor mediated and could involve cAMP-dependent protein kinase as well as G proteins. Since inhibition of the delayed rectifier K^+ current has been found to decrease the proliferative response in T lymphocytes, the β-adrenergic modulation of K^+ current may well serve as a feedback control mechanism limiting the extent of cellular proliferation.

Key Words K^+ channels · G proteins · adrenergic · cAMP

Introduction

Lymphocytes bear receptors for a wide variety of neurotransmitters and hormones that regulate cellular function through second messengers such as cyclic nucleotides (for review, *see* Coffey & Hadden, 1985; Plaut, 1987). The β-adrenergic receptor, which is stimulated by catecholamines, is of particular interest in that it may well be an important link between the autonomic nervous system and the immune system. The distribution of β-adrenergic receptors has been well characterized in human T lymphocytes (Khan et al., 1986). Catecholamines can influence lymphocyte proliferation (Vischer, 1976; Johnson, Ashmore & Gordon, 1981), antibody synthesis (Bourne et al., 1974; Miles et al., 1981; Hall et al., 1982; Sanders & Munson, 1984), and cytotoxic activity (Hatfield, Petersen & DiMicco, 1986). It has been shown that the responsiveness of lymphocytes to β-adrenergic agonists varies widely, depending on the type of cell and its state of maturation (Bach, 1975; Fuchs, Albright & Albright, 1988). Furthermore, the number of β-receptors in lymphocytes is modulated in certain diseases where immunologic abnormalities are implicated as playing a role in pathogenesis, e.g., β-receptor density is decreased on lymphocytes of asthmatic subjects (Meurs et al., 1982) and is upregulated in patients with multiple sclerosis (Arnason et al., 1988).

Voltage-gated K^+ currents have been implicated as being permissive in T-cell activation by mitogens (Chandy et al., 1984, 1985; DeCoursey et al., 1984, 1987; Deutsch, Krause & Lee, 1986; Lee et al., 1986). Based on these observations, we undertook these studies to determine (*i*) whether β-adrenergic stimulation modulates the voltage-dependent K^+ conductance in T lymphocytes and if so, (*ii*) whether the effect is mediated through cAMP. Whereas cAMP has been shown to decrease the voltage-dependent K^+ conductance in murine B lymphocytes (Choquet et al., 1987), comparable effects of intracellular cAMP have not been observed in T cells (Bregestovski, Redkozubov & Alexeev, 1986; Krause, Lee & Deutsch, 1988). We chose to study $CD8^+$ cells (suppressor/cytotoxic T cells) in view of recent findings demonstrating a higher density of β-adrenergic receptors on human $CD8^+$ cells as compared to $CD4^+$ cells (helper T cells) (Khan et al., 1986; Karaszewski et al., 1990). The maximal cAMP

generated in response to isoproterenol was likewise higher in suppressor/cytotoxic T cells than in helper T cells (Khan et al., 1985). We found that the β-agonist isoproterenol (*i*) decreased the amplitude of peak K^+ current, (*ii*) increased its rate of inactivation, (*iii*) decreased the voltage dependence of steady-state inactivation, and (*iv*) shifted the steady-state availability curve to the left. The effect appeared to be receptor mediated and cAMP dependent. In that block of the delayed rectifier current is associated with a decreased proliferative response in T lymphocytes (DeCoursey et al., 1984; Matteson & Deutsch, 1984); the β-adrenergic stimulus may well serve as a feedback control mechanism limiting the extent of cellular proliferation.

Materials and Methods

Isolation of $CD8^+$ Lymphocytes

Heparinized peripheral venous blood was obtained from healthy volunteers. Mononuclear cells were isolated using Lymphocyte Separation Mixture (Organon Teknika, Durham, NC) density gradient, washed three times with RPMI-1640 (Gibco, Grand Island, NY), and resuspended at 1×10^6 cells/cc in RPMI-1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) and 1% penicillin-streptomycin (Gibco). The mononuclear cell fraction was depleted of monocytes by selective adherence to tissue culture dishes for 1 hr at 37°C. The nonadherent cells were put into prepared B-cell panning dishes to remove B cells. Panning dishes were prepared by coating petri dishes for 2 hr with solutions containing either 10 μg/ml goat anti-human IgG (Organon Teknika) (B-cell pans) or goat anti-mouse IgG (Organon Teknika) (T-cell pans) according to the methods of Wysocki and Sato (1978). The T lymphocytes thus purified were then washed and incubated with OKT8 (10 μl per 1×10^7 cells) (Ortho Diagnostic Systems, Raritan, NJ) for 30 min at 0°C. $CD8^+$ labeled cells were added to the prepared T-cell pans for 2 hr prior to electrophysiological experiments. Cell adhesion with monoclonal antibodies, i.e., OKT8 was ascertained to be 98–99% specific using an indirect immunofluorescence assay, as described by Matteson and Deutsch (1984).

Electrophysiology

Current recordings were obtained using the whole-cell configuration of the patch-clamp technique as described by Hamill et al. (1981), using a List EPC-7 patch-clamp amplifier (List Electronic, Darmstadt/Eberstadt, West Germany). Recording pipettes were formed from soda lime glass (Blue-Dot Hematocrit Glass, Fisher Scientific, Pittsburgh, PA) using a vertical puller in a two-stage process. Pipette resistance ranged from 2–5 MΩ. The bathing solution consisted of the following (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, pH 7.3. The pipette (intracellular) solution contained (in mM): KCl 140, $CaCl_2$ 1, $MgCl_2$ 2, EGTA 11, HEPES 10, Mg-ATP 0.5, GTP 0.5, pH 7.2. The free Ca^{2+} of this solution was calculated to be 17 nM. In five control and six isoproterenol experiments, the pipette solution contained 70 mM KCl and 70 mM KF instead of 140 mM KCl. A bath perfusion system was employed in the external drug application experiments. Bath solution changes were made at the rate of 2 cc/min. Experiments were performed at room temperature (21–23°C).

An IBM-AT computer was used to acquire, store, and analyze the data. Voltage commands to the pipette were provided via the output of a Metrabyte D/A converter while the current data were sampled with a Data Translation DT2818 (Marlborough, MA) 12-bit A/D converter. Whole-cell current records were filtered at 2 kHz using an 8-pole Bessel filter and sampled at 5 kHz. In general, data were not leak- or capacity-transient corrected. The interpulse interval was sufficient to allow for recovery from the effect of the preceding pulse. Current decays were analyzed as a sum of exponentials by a Fourier method (Provencher, 1976) that determined the number, amplitudes, and time constants of the components. Nonlinear least squares were used for analyses fitting Boltzmann functions to the data.

Where three or more experiments were performed, values were reported as either the mean ± SEM or as the range with the number of experiments in parentheses. The statistical significance of the results was assessed by using a Student's *t* test and chi-square analysis.

The drugs used in this study were obtained from the following sources: (-)isoproterenol, cyclic adenosine 3′5′ monophosphate (cAMP), dibutyryl cAMP, D-L propranolol (Sigma, St. Louis, MO); cholera toxin (List Biological, Campbell, CA); protein kinase inhibitor (PKI_{5-24}) (Peninsula, Belmont, CA); and 8-(4-chlorophenylthio)-cAMP (Boehringer, W. Germany). The above drugs were dissolved in either distilled water or bathing solution and stored as stock solutions at −4°C. Solutions were prepared fresh from aliquots of stock solutions just before the experiments.

Results

Characterization of Outward Currents in $CD8^+$ Cells

Our experiments, in contrast to previous electrophysiological investigations of human T lymphocytes (Fukushima, Hagiwara & Henkart, 1984; Cahalan et al., 1985; Deutsch et al., 1986), were carried out on a subset-selected population of lymphocytes from peripheral blood. Depolarizing voltage pulses from a holding potential of −80 mV activated a voltage- and time-dependent outward current in $CD8^+$ lymphocytes which was carried predominantly by K^+ ions. The outward current had a reversal potential of -61 ± 3.2 mV ($n = 21$) as determined from tail current analysis and was sensitive to the K^+ channel blockers tetraethylammonium (TEA) and 4-aminopyridine (4-AP). We observed time-dependent changes in the kinetic properties of the voltage-dependent K^+ current over the first 10 min after establishing the whole-cell recording conformation, as has been previously reported for lymphocytes (Fukushima et al., 1984; Cahalan et al., 1985; Deutsch et al., 1986). The rates of activation and inactivation increased, while the peak currents elicited by a given

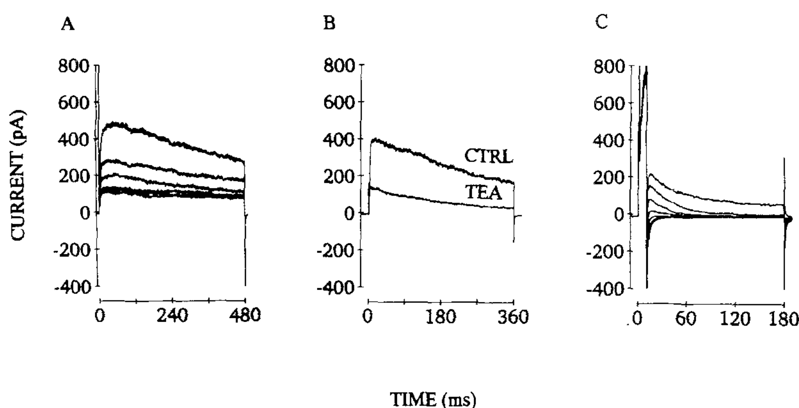

Fig. 1. Characterization of K^+ currents in human $CD8^+$ lymphocytes. (*A*) Cumulative inactivation of K^+ current during repetitive depolarization. Voltage steps of 480 msec duration were made to +40 mV from a holding potential of −80 mV at a rate of 0.5 Hz. (*B*) K^+ conductance inhibition by TEA. Pulses of 360 msec duration were made to +40 mV from the holding potential of −80 mV. Only current recordings obtained before and 10 min after perfusion with 10 mM TEA were shown. The amplitude of peak K^+ current was reduced by 60% following exposure to TEA. (*C*) Tail currents in $CD8^+$ cells. Ten-msec conditioning pulses to +40 mV from the holding potential of −80 mV were followed by pulses to more negative potentials from −30 to −100 mV in 10-mV steps. The time constant describing current deactivation ranged from 37.2 msec at −30 mV to 21.3 msec at −50 mV

depolarizing pulse decreased slightly with time following a variable initial increase. The membrane potential at which the outward current became observable usually shifted by approximately 10 mV more negative compared to the initial value.

Based on the presence of use-dependent inactivation, current sensitivity to TEA (10 mM), and the rate of K^+ channel closure, as determined from the decay of tail currents, the K^+ currents in $CD8^+$ cells appear to be of the *n* type as defined by DeCoursey et al. (1987). Data in support of this observation is presented in Fig. 1*A* which shows an example of K^+ current cumulative inactivation during repetitive depolarizations to +40 mV from a holding potential of −80 mV. Voltage stimuli (480-msec pulses) were delivered at 0.5 Hz. Figure 1*B* shows the current records obtained before and after perfusion with 10 mM TEA. The amplitude of the peak K^+ current was reduced by 60% following exposure to TEA. Tail currents were obtained using a standard double-pulse protocol in which brief depolarizations to +40 mV were followed by repeated steps to increasingly hyperpolarized potentials. The time course of tail current deactivation is a measure of the rate of K^+ channel closure and was determined from single-exponential fits to the current data at each potential. In the example shown in Fig. 1*C*, the time constant describing tail current decay ranged from 37.2 msec at −30 mV to 21.3 msec at −50 mV (exponential fits not shown).

Isoproterenol-Induced K⁺ Current Inhibition

Bath perfusion of voltage-clamped cells with the β-receptor agonist isoproterenol resulted in a decrease in the amplitude of peak voltage-dependent K^+ current in 13 of 16 experiments. In the remaining three experiments, current amplitude remained constant following isoproterenol application. The concentrations of isoproterenol used were 0.1 μM (n = 4), 1 μM (n = 10), and 10 μM (n = 2). No clear concentration-dependent effects were detected. In Fig. 2, a comparison is made between peak current amplitude and time course of inactivation at +40 mV obtained in response to isoproterenol perfusion *versus* that obtained in response to normal bath perfusion. The inhibitory effect of isoproterenol on peak current amplitude was observed throughout the voltage range, as depicted in the current records and corresponding current-voltage curves in Fig. 3. The time course of current inhibition from two representative cells is illustrated in Fig. 4*A*. The inhibitory effect of the addition of isoproterenol to the bath solution is clearly discernible when compared to variations in current amplitude over time alone in the control cell. Figure 4*B* is a summary of the time course of current inhibition in the 16 cells. The changes in peak current amplitude in each experiment were expressed as a percentage of the initial current amplitude at 5 min after establishing the whole-cell configuration in order to allow for equilibration. The results were then averaged to give the mean percent of initial current amplitude at 10, 20, and 30 min post whole cell.

Control experiments (*CTRL*) referred to here and subsequently, included three experiments where bathing solution, in the absence of drug, was perfused through the recording chamber to control for solution exchange artifacts and 12 experiments where changes in current amplitude and kinetics were observed in the absence of bathing solution exchange. All of these experiments lasted for at least 20 min after establishment of the whole-cell configuration. Six of the 15 control experiments were continued for an additional 10 min (30 min total). Because changes in K^+ currents occur between 5–10

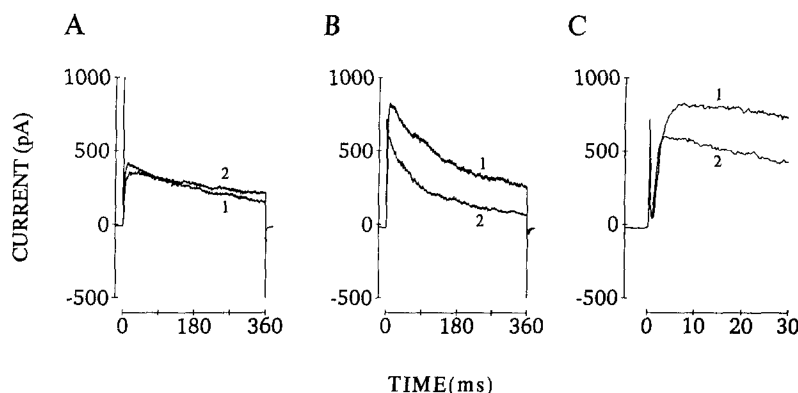

Fig. 2. Changes in lymphocyte K^+ current after perfusion with normal bath solution (*A*) and after perfusion with isoproterenol (*B*). Pulses of 360 msec duration were stepped to +40 mV from a holding potential of −80 mV at 60-sec intervals. *1* = before perfusion. *2* = 10 min after perfusion. Note that peak current amplitude was reduced and the rate of current inactivation enhanced in the presence of isoproterenol. (*C*) Resolution of the peak current from the same cell as in *B*, showing clear separation of the peak current from the capacity transient

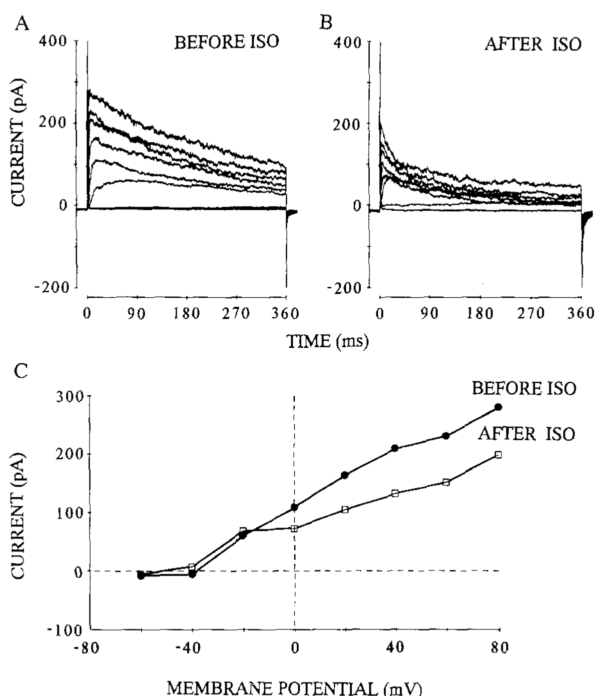

Fig. 3. Effect of isoproterenol (*ISO*) on K^+ current at various potentials in $CD8^+$ lymphocytes. Whole-cell current records from the same cell are shown before (*A*) and 10 min after perfusion with 1 μM isoproterenol (*B*). Pulses of 360 msec duration delivered at 30-sec intervals were stepped from −80 mV to the following potentials in mV: −60, −40, −20, 0, 20, 40, 60, and 80. (*C*) The corresponding current-voltage curve constructed from the peak currents

min post whole cell, perfusion of isoproterenol was performed only after 10 min post whole cell and monitored for 10 min following perfusion. Eight of these 16 experiments were continued for an additional 10 min (20 min total following perfusion of 30 min post whole cell). As shown in Fig. 4*B*, an average reduction of 24.1 ± 5.2% (n = 16) in the peak K^+ current amplitude was observed at 10 min after perfusion with isoproterenol (20 min post whole cell). Current inhibition continued to develop, reaching a 45.6 ± 4.9% (n = 8) reduction at 20 min after perfusion (30 min post whole cell). In control experiments, a slight reduction (10%) in the amplitude of the currents was evident at 30 min following establishment of whole-cell configuration. Perfusion with normal bath solution in three of the control experiments likewise did not decrease the current amplitude (percentage change was 1.7 ± 6.6% at 10 min following perfusion). The results were similar regardless of whether the pipette filling solution contained 140 mM KCl or 70 mM KCl and 70 mM KF.

The possibility that the effects of isoproterenol could be due to the activation of an inward current was also examined. Outward current was blocked by isotonically replacing the K^+ in the pipette with Cs^+. In the presence of internal Cs^+, isoproterenol failed to induce inward current activation (n = 2). Therefore, the reduction in the amplitude of the outward currents cannot be ascribed to an increase in inward current.

Effect of Isoproterenol on the Kinetics of Outward Current Activation and Inactivation

The time course of current inactivation was well described as a single-exponential process in 9 of 15 control experiments and 11 of 16 isoproterenol experiments; however, it could be best fitted with two exponentials in 4 of 15 control and 3 of 16 isoproterenol experiments. In two control and two isoproterenol experiments, the initial current record could not be well fitted and these records were, therefore, excluded in the time constant analysis of current decays. The initial time constant describing current decay determined at 5 min post whole cell for the remaining 27 cells ranged from 175–757 msec. In the experiments where current inactivation was best fitted with two exponentials, an additional faster time constant ranging from 6.5–77 msec was pres-

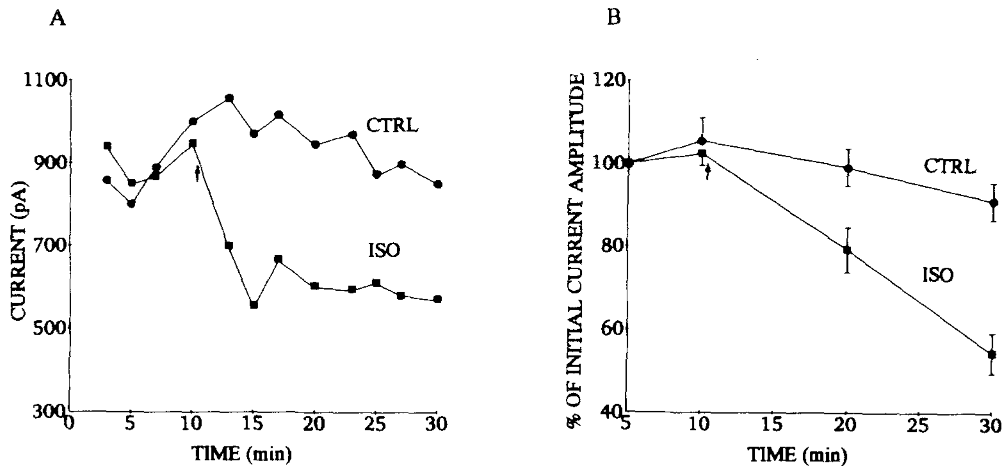

Fig. 4. Comparison of the effects of isoproterenol (*ISO*) on K^+ current amplitude with time-dependent changes in peak current amplitude in the absence of agonist (*CTRL*). (*A*) Representative examples of control (*CTRL*) and isoproterenol experiments are shown. The initiation of isoproterenol (0.1–1.0 μM) perfusion is indicated by the arrow. (*B*) Summary of the time course of current inhibition is shown. The changes in peak K^+ current amplitude in each experiment were expressed as a percentage of the initial current amplitude taken at 5 min after the establishment of the whole cell. The results were then averaged to give the mean percent of initial current amplitude ±SEM at 10, 20, and 30 min post whole cell. The number of experiments in the control experiments was 15, 6 of which were monitored for up to 30 min. There were a total of 16 cells in the isoproterenol studies; 8 of these experiments were followed for up to 30 min

ent. Since there was no consistent isoproterenol-induced change in the fast time constant (when present), only changes in slow time constant of current inactivation were considered in the kinetic analysis of the data. Figure 5*A* illustrates an example of exponential fits to the decay phase of the current before and 10 min after perfusion with isoproterenol (1 μM). Due to a wide variability in the time constants of inactivation from cell to cell, the changes in the rate of current inactivation were normalized in each experiment and expressed as a percentage of the initial time constant taken at 5-min post whole-cell formation. As shown in Fig. 5*B*, the time constant of inactivation decreased by 15–25% at 10-min post whole-cell formation. In control experiments, it stabilized over the next 20 min. In contrast, perfusion with isoproterenol resulted in an additional decrease in the time constant; the percent decrease in the time constant of inactivation at 10 min after perfusion (20 min post whole cell) was 52.9 ± 2.8% (n = 14), as compared to 15.3 ± 7.6% (n = 13) in control experiments ($P < 0.005$ as determined by the Student's t test). Determination of significance using chi-square analysis yielded similar results; 2 of 13 control experiments and 12 of 14 isoproterenol experiments showed a reduction in the time constant of current inactivation of greater than 40% ($P < 0.01$). The effect of isoproterenol on both current amplitude and time course was not reversible with repeated washing over a 15–20 min period.

Isoproterenol had no effect on the time course of current activation. The time course of current activation, estimated as the time to peak, was observed to decrease with time in both the control and isoproterenol experiments (Table 1). Recovery from inactivation was examined by observing the current produced by a test voltage pulse delivered at varying intervals after a conditioning voltage pulse of the same amplitude. The time constant of recovery was 18.5 ± 1.5 sec for a 380-msec pulse in control experiments (n = 7). A slower component of recovery may be present but could not be resolved accurately. In three experiments where recovery in the presence of isoproterenol was assessed, the time constant decreased by 22–64%. However, the same effect was observed with time alone. Similarly, a slowing of the channel closing rate as determined from exponential fits to tail currents seen after isoproterenol perfusion (n = 3) could not be distinguished from the change observed in control experiments (n = 3) with time.

Effect of Isoproterenol on the Steady-State Parameters of Activation and Inactivation

The steady-state parameters of K^+ current activation were determined from conductance-voltage ($g_K - V$) curves which were fitted with a Boltzmann function of the form:

$$g_K(V) = g_{K,\max}/[1 + e^{(V-V_n)/k_n}] \qquad (1)$$

where V_n is the voltage at the midpoint of the activa-

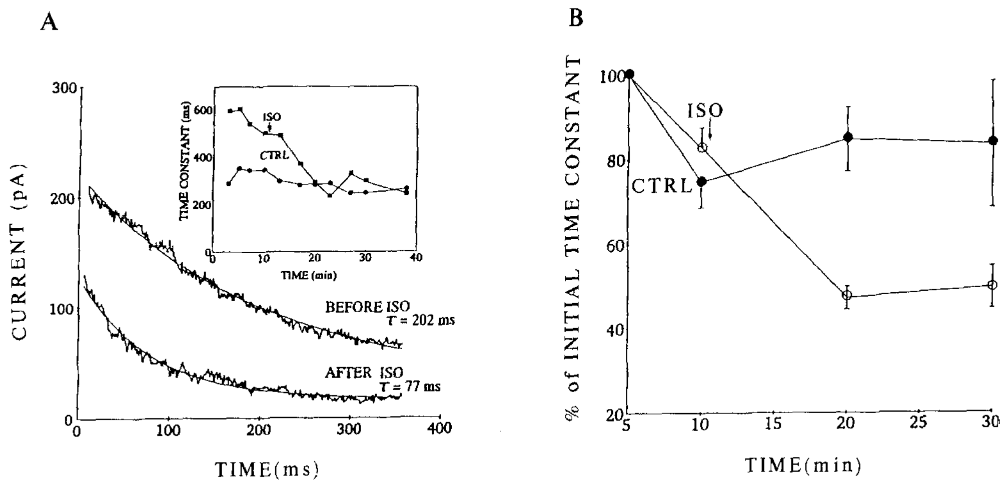

Fig. 5. Effect of isoproterenol on the time course of K^+ current inactivation from a single cell. A depolarizing pulse of 360 msec duration was stepped to +40 from −80 mV. (*A*) The exponential fit to the data points is superimposed on each current record (before perfusion and 10 min after perfusion with isoproterenol). Isoproterenol (1 μM) decreased the time constant of inactivation by 60%. The inset illustrates the time-dependent changes in the time course of inactivation in a control and in an isoproterenol experiment. (*B*) Summary of the data on the time constant of inactivation. The changes in the time constant of K^+ current inactivation were expressed as a percentage of the initial time constant taken at 5 min after establishment of the whole-cell configuration. The results were then averaged to give the percent of initial time constant ±SEM at 10, 20, and 30 min post whole cell. The initiation of the isoproterenol perfusion is indicated by the arrow

tion curve and k_n is the steepness of the voltage dependence. In control experiments, there was a 8.8 ± 0.9 mV (n = 7) negative shift in the voltage at which the conductance was half-maximal ($V_{1/2}$) at 20 min post whole-cell formation. The shift of $V_{1/2}$ toward a more negative potential in the isoproterenol experiments was 12.2 ± 2.2 mV (n = 7) at 10 min following exposure to isoproterenol (also 20 min following whole-cell formation). This difference was not statistically significant. There was similarly no significant change in the slope factor. A summary of the steady-state parameters of current activation with time alone and in the presence of isoproterenol is given in Table 2. Shifts of similar magnitude in the threshold potential over time have been reported by other investigators (Deutsch et al., 1986; Fukushima et al., 1984).

Due to the slow recovery from inactivation, steady-state inactivation as a function of membrane potential was determined by varying the holding potential for a period of 2 min prior to recording the K^+ current at a constant test potential of +40 mV. The normalized peak currents were likewise fitted by a Boltzmann function:

$$j = I_{K,\max}/[1 + e^{(V-V_j)/k_j}] \tag{2}$$

where V_j is the voltage at which the current is half-inactivated and k_j the slope factor. The subscripts n and j in Eqs. (1) and (2) represent activation and inactivation parameters, respectively. In 6 of 14 control cells studied, the current at −60 mV was at least 40% inactivated when compared to values obtained at −100 mV. The rest of the cells showed no appreciable inactivation in the range from −60 to −100 mV. Table 3 summarizes the data from five control cells where steady-state availability curves were measured both at 5 and 20 min after establishment of whole-cell configuration. There was minimal shift in the $V_{1/2}$ and k_j during this time period. In contrast, isoproterenol altered the voltage dependence of the steady-state availability curve, producing a hyperpolarizing shift in the midpoint of inactivation of 6.7 ± 1.5 mV and increasing the slope factor by 3.8 ± 1.2 (n = 6; $P < 0.05$). Since the cells did not tolerate holding potentials more negative than −100 mV, saturation of the curve could not be obtained in the presence of isoproterenol. Considering this, the magnitude of the shift in the midpoint of steady-state inactivation is then, an estimate of the total shift. Examples of steady-state availability curves for two representative experiments are depicted in Fig. 6 where continuous lines represent the Boltzmann fit to the data.

Isoproterenol-Induced Current Inhibition is Receptor Mediated and cAMP Dependent

Pre-exposure of the cells to the β-blocker propranolol at a concentration of 10 μM (for 30 min in five experiments and for 90 min in four experiments)

Table 1. Activation time course in control and isoproterenol experiments

	Control (n = 15)	Isoproterenol (n = 16)	P value
	(msec)	(msec)	
5 min	12.8 ± 2.6	14.7 ± 2.8	
20 min	8.1 ± 1.9	9.5 ± 2.2	
Mean difference	4.7 ± 1.4	5.1 ± 1.9	$P > 0.05$

Time values in column 1 refer to time after establishing the whole-cell configuration. Activation time course was measured as time to peak current.

Table 2. Steady-state parameters of activation

Condition	V_n A (5 min)	V_n B (20 min)	V_n B − A
CTRL (n = 7)	−24.0 ± 2.3	−33.2 ± 3.1	−8.8 ± 0.9
Iso (n = 7)	−23.9 ± 2.6	−36.2 ± 2.8	−12.2 ± 2.2
P value			$P > 0.05$

Condition	k_n A (5 min)	k_n B (20 min)	k_n B − A
CTRL (n = 7)	−5.2 ± 0.6	−4.6 ± 0.3	0.5 ± 0.3
Iso (n = 7)	−5.2 ± 0.4	−3.7 ± 0.5	1.3 ± 0.8
P value			$P > 0.05$

The voltage dependence of steady-state activation of K^+ currents was obtained from conductance-voltage curves which were fitted with a Boltzmann function, allowing the determination of the midpoint (V_n) and the slope factor (k_n). Time values within the parentheses refer to time after formation of the whole-cell configuration. In the isoproterenol experiments, perfusion with isoproterenol was performed after 10 min post whole-cell. P values were determined by Student's t test.

inhibited the effect of isoproterenol on peak current amplitude but only partially attenuated the change in time course of inactivation. In cells pretreated with propranolol, isoproterenol decreased the time constant of inactivation by 42.9 ± 9.2% of the initial value, as compared to a decrease of 52.9 ± 2.8% observed in the untreated cells. These results are summarized in Fig. 7.

Perfusion of the cells with 1 mM dibutyryl cAMP decreased the amplitude of peak K^+ current by 20 ± 4.7% (n = 10) and decreased the time constant of current inactivation by 54 ± 5.7% at 10 min post-perfusion. At 20-min post perfusion, peak current amplitude was decreased by 42.1 ± 9.2% (n = 6), while no further reduction in the time constant of current inactivation was observed. Figure 8*A* illustrates the effect of 1 mM dibutyryl cAMP on K^+ current amplitude and kinetics. In contrast, when either 1 mM cAMP plus 1 mM theophylline or 8-(4-chlorphenylthio)-cAMP (50 μM), a nonhydrolyzable cAMP analogue, were added to the intracellular solution, there was no significant change in peak current amplitude or the kinetics of activation and inactivation at 10 min (n = 13) or 20 min (n = 4) after whole-cell formation when compared to control experiments.

When the protein kinase A inhibitor ($PKI_{5\text{-}24}$), a 20 amino acid-residue synthetic peptide that inhibits cAMP-dependent protein kinase (Cheng et al., 1985), was added to the pipette solution at concentrations of 2–5 μM, the effect of isoproterenol on the amplitude of K^+ current was partially attenuated. Figure 8*B* illustrates an example of whole-cell current records obtained before and after perfusion of the voltage-clamped cell with isoproterenol in the presence of PKI (5 μM) in the pipette solution. The results of these experiments were averaged in Fig. 8*C* and compared to isoproterenol experiments in the absence of internal PKI and to experiments with PKI alone. In the presence of PKI alone, mean current amplitude at 30 min following whole-cell formation was 103 ± 4.6% (n = 4) of the initial current amplitude, as compared to 90.9 ± 4.5% (n = 6) in the control experiments ($P < 0.05$). Thus, the presence of PKI in the pipette solution decreased the washout of the current which was observed with time alone in the control experiments, suggesting that there is a basal level of protein kinase A mediated channel phosphorylation (current inhibition) in the absence of extrinsic β-adrenergic stimulation. Perfusion with isoproterenol (1 μM) in the presence of intracellular PKI resulted in 10.7 ± 4.7% (n = 9) decrease in peak current amplitude and a 40 ± 6% (n = 9) decrease in the time constant of current inactivation at 10 min following perfusion. Internal PKI was more effective in inhibiting the isoproterenol-induced decrease in current amplitude than the decrease in the time constant of current inactivation which suggested that (*i*) the two kinetic parameters might be modulated independently and (*ii*) the presence of a modulatory mechanism in addition to a cAMP-dependent phosphorylation event.

The response to isoproterenol was mimicked following perfusion of the cell with cholera toxin (1 μg/ml) presumably through the cholera toxin-sensitive G protein, G_s. The amplitude of the currents decreased by 10–25% and the rate of inactivation was increased by 50–80% in the presence of cholera toxin (n = 3; *data not shown*). When 300 μM GTP-γ-S, a nonhydrolyzable GTP analog, was included

Table 3. Steady-state parameters of inactivation

Condition	V_j		
	A (5 min)	B (20 min)	B − A
CTRL (n = 5)	−53.2 ± 2.5	−54.9 ± 2.1	−1.8 ± 1.0
Iso (n = 6)	−54.5 ± 2.1	−61.2 ± 3.2	−6.7 ± 1.5
P value			$P < 0.05$

Condition	k_j		
	A (5 min)	B (20 min)	B − A
CTRL (n = 5)	5.9 ± 0.9	5.1 ± 0.6	−0.8 ± 0.5
Iso (n = 6)	5.0 ± 0.6	8.8 ± 1.4	3.8 ± 1.3
P value			$P < 0.05$

The voltage dependence of steady-state inactivation was determined as described in the text. Normalized peak K^+ currents were fitted with a Boltzmann function allowing the determination of the midpoint (V_j) and the slope factor (k_j). Time values within the parentheses refer to time after formation of the whole-cell configuration. In the isoproterenol experiments, perfusion with isoproterenol was done after 10 min post whole-cell. P values were determined by Student's t test.

in the pipette solution, the peak current amplitude did not change significantly at 10 min following whole-cell formation (n = 8), but decreased by 15.8 ± 4.50% (n = 5) at 15–20 min. The time constant describing current inactivation decreased by 46 ± 11.3% (n = 6) and 55 ± 12%, respectively, during the same time period. Thus, the effects of both cholera toxin and intrapipette GTP-γ-S on the time course of current inactivation were more prominent when compared to the effects on peak current amplitude.

Discussion

Results from these studies demonstrate that β-adrenergic stimulation modulates voltage-dependent K^+ currents in subset-selected peripheral blood lymphocytes. The β-receptor agonist isoproterenol decreased K^+ current amplitude and enhanced the rate of current inactivation in $CD8^+$ lymphocytes. Isoproterenol, in addition, decreased the voltage dependence of steady-state K^+ current inactivation and shifted the steady-state availability curve to the left. The effects of the β-agonist were receptor mediated and appear to involve G proteins as well as the cAMP-dependent protein kinase. The isoproterenol-induced decrease in current amplitude was inhibited by the β-blocker propranolol. The isoproterenol-induced decrease in the time constant of current inactivation was, however, only partially inhibited in cells preincubated with propranolol. The reduction in peak current amplitude as well as the enhancement of current inactivation was mimicked by membrane-permeant analogues of cAMP. The reduction of peak current amplitude in response to isoproterenol was attenuated when a synthetic peptide inhibitor of the cAMP-dependent protein kinase (PKI_{5-24}) was present in the pipette solution. Internal concentrations of the nonhydrolyzable GTP analogue, GTP-γ-S, also enhanced current inactivation as did exposure of the cell to cholera toxin implicating the possibility of G protein involvement in the response.

Receptors for a variety of hormones and neurotransmitters have been characterized on both murine and human lymphocytes (*see* Coffey & Hadden, 1985; Plaut, 1987). An increased responsiveness to histamine, prostaglandins, and β-adrenergic agonists has been observed to occur as a late-stage event further demonstrating an important role played by these agents in immunoregulation (Dailey, Schreurs & Schulman, 1988). Hormones and neurotransmitters are involved in the modulation of K^+ currents, as illustrated recently in a murine pre-B lymphocyte cell line where serotonin (5-HT) appeared to increase the maximum conductance due to the activation of 5-HT_1 receptors and to accelerate the time course of inactivation through the activation of 5-HT_3 receptors (Choquet & Korn, 1988). In Jurkat E6-1 human T lymphocytes, substance P was found to reduce K^+ current peak amplitude and to accelerate current inactivation via a GTP-binding protein (Schumann & Gardner, 1989).

Krause et al. (1988) were unable to demonstrate isoproterenol-induced modulation of the voltage-gated K^+ conductance in human T cells. The studies of Krause and co-workers compared mean current amplitudes in lymphocytes preincubated with isoproterenol for 30 min to 3 hr to mean peak current values obtained in controls. Studies described in this investigation differed from those of Krause et al., (1988) in that (*i*) experiments were performed on a subset of lymphocytes reported to maximally express β-receptors, and (*ii*) current data was obtained prior to and following β-adrenergic stimulation on the same cell.

Investigations of homologous desensitization of β-adrenergic receptors have demonstrated receptor downregulation or the occurrence of sequestration at cytoplasmic sites distant from the cell surface after a lag of 30–60 min following agonist exposure. Receptor sequestration occurs in addition to an uncoupling of the receptor-adenylate cyclase interaction that takes place within minutes (for review, *see* Lefkowitz, Stadel & Caron, 1983; Hertel & Perkins,

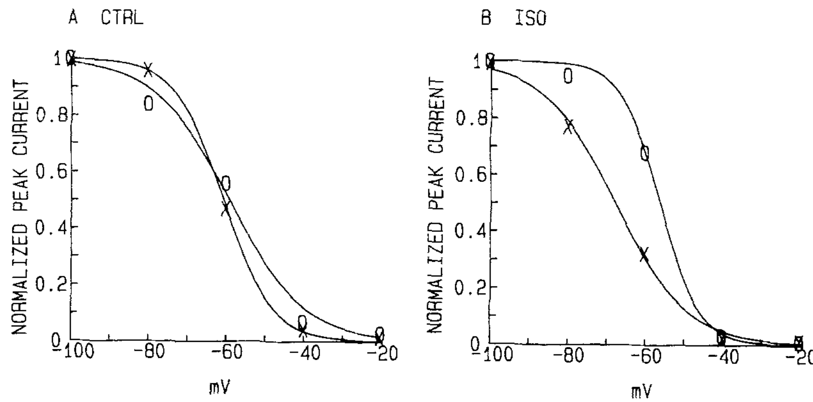

Fig. 6. Steady-state parameters of inactivation in control and isoproterenol experiments from two representative cells. Voltage-dependence of inactivation was determined by varying the holding potential and recording the K^+ current at +40 mV after several minutes. The normalized data points were fitted with a Boltzmann function (Eq. (2)). (*A*) Control experiment. V_j = −58.4 mV; k_j = 9.1 at 5 min (circles); V_j = −60.8 mV; k_j = 6.4 at 20 min post whole cell (crosses). (*B*) Isoproterenol experiment. V_j = −56. mV; k_j = 5.5 before perfusion (circles); V_j = −68.8 mV; k_j = 8.8 after perfusion (crosses)

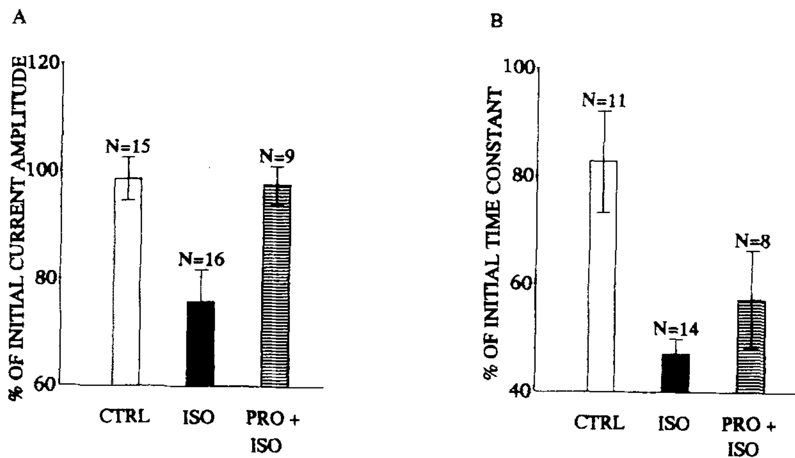

Fig. 7. Summary of the effect of isoproterenol (*ISO*) and propranolol (*PRO*) experiments on the amplitude and time course of inactivation of K^+ currents in lymphocytes. The response to isoproterenol in each experiment is expressed as the ratio of either peak current amplitude or the time constant describing current decay at +40 mV measured at 10 min after perfusion (20 min post whole cell) to values before perfusion (5 min post-whole cell) × 100%. (*A*) Isoproterenol (0.1–10 μM) decreased the current amplitude to 75.8 ± 5.9% (n = 16) of the initial value. Preincubation with propranolol (1–10 μM) for 0.5–1.5 hr inhibited the response to isoproterenol. Statistical significance: *CTRL vs. ISO* = $P < 0.005$; *ISO vs. PRO + ISO* = $P < 0.01$. (*B*) The time constant describing current decay was decreased to 47.1 ± 2.8% (n = 14) of the initial value in the presence of isoproterenol, as compared to 82.7 ± 9.4% (n = 11) observed with time alone. Propranolol only partially attenuates the effect of isoproterenol on the time course of inactivation. Statistical significance: *CTRL vs. ISO* = $P < 0.001$; *ISO vs. PRO + ISO* = $P > 0.05$. Statistical analysis was performed using Student's t test

1984) following agonist exposure. Perfusion experiments using individual cells as their own controls, as opposed to preincubation experiments, minimized the risk of agonist-induced desensitization and maximized the detection of any isoproterenol-induced alteration considering the wide variability in the current expression from cell to cell.

The isoproterenol-induced modulation of K^+ current in lymphocytes may result from: (*i*) K^+ channel phosphorylation by cAMP-dependent protein kinase and/or by other protein kinases, (*ii*) direct G-protein gating of the ion channel as has been reported for the adrenergic modulation of cardiac Na^+ and Ca^{2+} channels (Schubert et al., 1989; Yatani & Brown, 1989), or finally, (*iii*) an increase in cytosolic free Ca^{2+} possibly through stimulation of some Ca^{2+} influx or release pathway, since K^+ outward currents in lymphocytes have been shown to be inactivated by elevated intracellular Ca^{2+} concentrations (Bregestovski et al., 1986; Choquet et al., 1987). Since the β-blocker, propranolol inhibited the isoproterenol-induced decrease in peak current amplitude, it is reasonable to conclude that the isoproterenol effect is receptor mediated. Our results concur with the findings of Krause et al. (1988) and Bregestovski et al. (1986) that addition of cAMP to the intracellular solution does not have any significant effect on K^+ current kinetics. However, bath application of a membrane-permeant cAMP analogue mimicked the effects of isoproterenol on K^+

current amplitude and kinetics. The apparent discrepancy between the effects of extracellular *versus* intracellular cAMP could have two possible explanations. It is possible that cAMP derivatives exert an extracellular effect that is separate from their intracellular effect as second messengers (for review, *see* Tsien, 1977). Alternatively, diffusional access of cAMP from the pipette to the cell interior could have been impeded, and therefore, the chances of successfully getting cAMP into the cell at higher concentrations might be increased by external application of membrane-permeant analogues. Nevertheless, the finding that PKI_{5-24} in the pipette solution attenuated the reduction in peak current amplitude in response to isoproterenol is consistent with the hypothesis that isoproterenol is, in fact, acting via cAMP. It has been shown that this peptide exhibits a high degree of specificity for cAMP-dependent protein kinase, even though the arginine-cluster basic subsite essential for binding to catalytic subunit appears to be a specificity determinant for several other enzymes (Cheng et al., 1986).

Both cholera toxin and GTP-γ-S exerted a more striking effect on the time course of inactivation than on peak current amplitude. In addition, the presence of PKI_{5-24} in the pipette solution did not completely inhibit the modulatory effects of isoproterenol. While these findings suggest that a G protein is involved in the action of isoproterenol, a direct G protein effect cannot be ascertained definitively, short of single-channel studies using inside-out patches and exposing the cytoplasmic surface to activated G_k, G_o, or G_s. One must also be cautious in the interpretation of these data in view of the recent findings that the β-subunit of cholera toxin as well as whole-cholera toxin can induce rapid and sustained increases in cytoplasmic Ca^{2+} (Dixon et al., 1987; Maenz, Gabriel & Forsyth, 1987). That isoproterenol may exert its effect by an increase in intracellular Ca^{2+} is possible, although studies have shown that agents that increase cAMP antagonize the Con A mediated increase in cytosolic free Ca^{2+} (Tsien, Pozzan & Rink, 1982) in lymphocytes. Furthermore, one would assume that the pipette solution which contains 1 mM Ca^{2+} and 11 mM EGTA, would effectively buffer any increase in Ca^{2+} in small cells like lymphocytes (6–8 μm in diameter).

In addition to K^+ channels, Cl^- channels have recently been characterized in T lymphocytes, and these can be induced to open following either a prolonged period of depolarization, by the presence of an osmotic gradient across the membrane, by increased intracellular levels of cAMP, or by an increase in the internal free Ca^{2+} level to 10^{-7} M (Cahalan & Lewis, 1988; Chen, Schulman & Gardner, 1989). It is conceivable that agents that elevate intracellular cAMP could also activate Cl^- current as has been reported for Jurkat E6-1 T lymphocytes (Chen et al., 1989); however, this will only result in an underestimation of the β-adrenergic effect on K^+ currents.

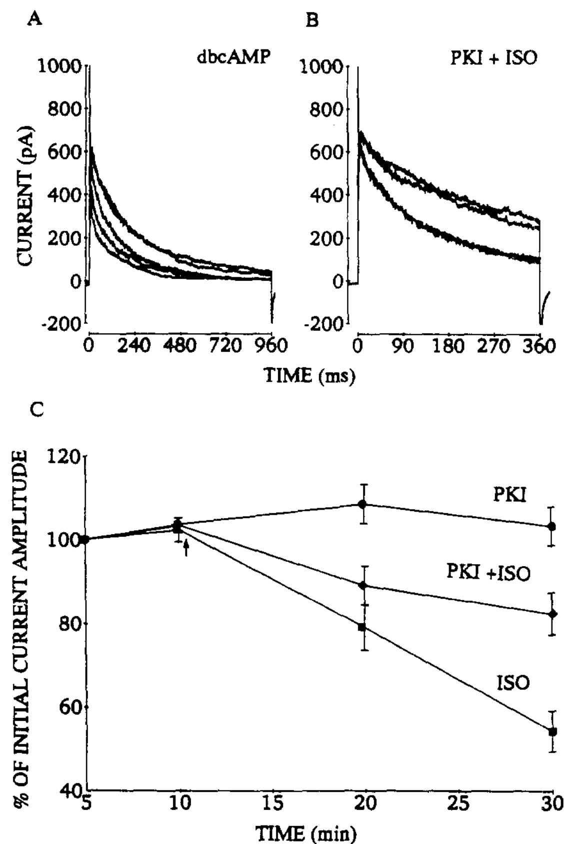

Fig. 8. Effect of cAMP and PKI on K^+ currents in lymphocytes. Depolarizing pulses were stepped from −80 to +40 mV at 60-sec intervals. Pulse duration was 960 msec in *A* and 360 msec in *B*. (*A*) Examples of whole-cell currents recorded at 5 and 10 min post whole cell (top two pulses shown) and at 5, 10, and 15 min following perfusion with 1 mM dbcAMP (bottom three pulses shown). There was a 28% decrease in the peak current amplitude and 38% reduction in the time constant describing current decay phase at 10 min following perfusion. (*B*) Examples of whole-cell currents recorded at the same time period as shown in *A* with 5 μM PKI_{5-24} added to the intracellular solution and isoproterenol (1 μM) perfused at 10 min post-whole cell. Top two pulses were 5 and 10 min post whole cell and bottom three pulses (overlapped) were 5, 10 and 15 min following perfusion with isoproterenol. (*C*) Comparison of the effects of isoproterenol on the amplitude of K^+ currents in the presence and in the absence of PKI_{5-24} (2–5 μM) in the pipette solution: (*i*) *PKI* alone ($n = 4$); (*ii*) *PKI + ISO* ($n = 9$), five of these experiments were continued for up to 30 min post whole cell; and (*iii*) *ISO* alone ($n = 16$), eight of these experiments were continued for up to 30 min post whole cell

The fact that K^+ current in lymphocytes can be modulated by isoproterenol is intriguing in view of the studies demonstrating that K^+ channel blockers

inhibit T-cell proliferation (Chandy et al., 1984; Lee et al., 1986; DosReis, Nobrega & Persechini, 1988). However, whether the opening of K^+ channels is critical for T-cell activation and whether the inhibitory effects of K^+ channel blockers are specific for signal transduction events initiated through the antigen-receptor remain unresolved (Gelfand et al., 1987; Schell et al., 1987). Nonetheless, β-adrenergic modulation of K current in lymphocytes may serve to limit the magnitude and extent of cellular activation and proliferation.

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