Biochemical Pharmacology, Vol. 52, pp. 843–849, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00350-4

Propofol Blocks Voltage-Gated Potassium Channels in Human T Lymphocytes

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ABSTRACT. The effect of propofol (PR) on voltage-gated potassium channels (K_V) in human T lymphocytes (TL) was studied using the patch-clamp technique in the whole-cell configuration. PR was found to reversibly block the K_V channels in a dose-dependent manner with a half-blocking concentration of approximately 40 μM. The decrease in the peak current caused by PR was voltage-independent. The activation time constant of the whole-cell potassium currents remained unaffected upon PR treatment, whereas both the rate and extent of the inactivation process were increased, indicating the "open channel block" mechanism. The PR half-blocking concentration was of the same order of magnitude as PR blood concentrations employed in anesthesia. Taking into account the extensive use of PR and the important role of K_V channels in human TL, these results suggest a need for investigations into the effect of PR on TL cell-function regulation. BIOCHEM PHARMACOL 52;6:843–849, 1996.

KEY WORDS. propofol; potassium channels; T lymphocytes

PR† (2,6-diisopropylphenol), a strongly hydrophobic substituted phenol, is an intravenous anesthetic used for both induction and maintenance of general anesthesia [1, 2]. Despite its clinical usefulness, little is known about the molecular mechanism of PR action. On the other hand, electrophysiological studies have provided evidence that PR acts on various ionic channels. It has been demonstrated that PR strongly potentiates inhibitory synaptic transmission by enhancing the activity of $GABA_A$ chloride channels [3, 4], inhibits sodium channels in human brain cortex [5], affects sodium and potassium currents underlying the action potential in frog myelinated axons [6] and inhibits calcium currents in guinea-pig isolated ventricular myocytes [7]. PR has also been shown to block voltage-gated potassium channels in canine myocardial cells [8], in PC12 cells [9], and in pig articular chondrocytes [10].

Several lines of evidence indicate involvement of potassium channels in the control and modulation of cell functions in nonexcitable cells [11]. A large body of experimental observations on the role of potassium channels in cell-function regulation in nonexcitable cells came from experiments on TL. Available data provide evidence that ("inactivating delayed rectifiers") and K_{Ca} channels are present in TL plasma membrane [12–15]. However, it has been demonstrated that the K_V channels are predominant in resting human TL [12,16–20]. It has been shown that K_V channels play an important role in setting the TL membrane resting potential [21, 22]. Moreover, it has been demonstrated that these channels are involved in controlling processes such as TL mitogenesis [11, 13, 14, 23–27] and volume regulation [21, 28].

Taking into account the wide use of PR and the fact that such intravenous drugs may directly interact with blood cells, it is of interest to investigate the effect of PR on the potassium channels in TL. In the present paper, we show that PR reversibly blocks K_V channels in human TL with a half-blocking concentration of approximately 40 μM, which lies within the range of concentrations used in anesthesia. Analysis of the whole-cell potassium currents provides evidence for an open channel block mechanism.

MATERIALS AND METHODS

Cell Separation and Culture

Human TL were separated from peripheral blood samples from 4 healthy donors using a standard method described elsewhere [29]. After separation, cells were cultured in Heraeus Incubator (Heraeus Instruments, Hanau, Germany) for 1–3 days in plastic Petri dishes in RPMI 1640 medium (Sigma) containing 5% (v/v) horse serum. Before electrophysiological experiments, cells were centrifuged at 600 *g*

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† *Abbreviations:* PR, propofol; TL, human T lymphocytes; K_V, voltage-gated potassium channels; K_{Ca}, Ca^{2+}-activated potassium channels; I_{max}, amplitude of the measured current; τ_n, time constant of activation; *p*, the exponent of the activation term; *h*, steady-state fraction of active channels; τ_h, time constant of inactivation.

Received 11 October 1995; accepted 19 April 1996.

for 5 min and then placed in the external solution (see below). The experiments were performed on the cultured cells for at least 24 hr.

Propofol

Propofol (Lancaster, France), technical grade, was purified by distillation under reduced pressure (3 times) and crystallization from hexane. Purity of PR was assessed by nuclear magnetic resonance. PR was then dissolved in DMSO at a concentration of 50.2 mM and stored at 4°C.

Electrophysiological Recordings

Potassium currents were recorded in the whole-cell configuration of the patch-clamp technique [30], using an EPC-7 amplifier (List Electronics, Darmstadt, Germany). Electrophysiological experiments were performed in a bathing saline (in mM: 150 NaCl, 4.5 KCl, 1 $CaCl_2$. 1 $MgCl_2$. 10 HEPES, 10 Glucose, pH = 7.35) under a phase contrast illumination using the inverted Olympus IMT-2 microscope. Patch pipettes were pulled from borosilicate glass (Hilgenberg, Germany) and fire-polished immediately before the experiment. The pipettes were filled with an internal solution (in mM: 70 KF, 80 KCl, 1 $CaCL_2$, 1 $MgCl_2$, 10 HEPES, 10 EGTA, pH = 7.2). Similarly to what Cahalan *et al.* observed [17], the presence of F^- in the internal solution improved the stability of the whole-cell configuration. The pipette resistance was in the range of 3–5 MΩ. Effects of the whole-cell capacitance and series resistance were compensated by using the proper compensation circuits of the amplifier. The access (series) resistance, estimated by setting the parameters for cancellation of the whole-cell capacitance transients, was typically in the range of 10–20 MΩ. The current signals were low-pass filtered at 3 kHz, digitized using the LAB MASTER TL-1 (Axon Instruments, USA) analog-to-digital converter with the sampling rate of 10 kHz, and stored on the computer hard disk. A standard protocol of voltage stimuli was applied (holding potential = −90 mV, 7 pulses from −60 to +60 mV with 20 mV increment applied every 30 sec, pulse duration −50 ms). The P/4 procedure was used to provide on-line cancellation of any linear (ohmic) component of the recorded currents. Before onset of each depolarizing pulse, a series of 4 hyperpolarizing prepulses was applied. Amplitude of each prepulse was ¼ of the following depolarizing pulse. Currents recorded when applying the prepulses were summarized and added to a current recorded during the depolarizing pulse.

The recorded whole-cell currents were fitted with the classical Hodgkin-Huxley equation in the form:

$$I(t) = I_{max}\,(1-\exp(-t/\tau_n))^p(h-(h-1)\exp(-t/\tau_h)) \qquad (1)$$

where I_{max} is the amplitude of the measured current, τ_n is the time-constant of activation, p is the exponent of the activation term, h is the steady-state fraction of active channels, and τ_h is the time-constant of inactivation.

Unless otherwise stated, data are given as mean ± standard error (SE). All experiments were carried out at room temperature (21–23°C).

Statistical Analysis

Unless otherwise stated, data were analyzed by applying the one-way analysis of variance (ANOVA). Both the F, which is a calculated value of the test statistics, and the P values are given. The differences in measured parameters were considered statistically significant when the P value was less than 0.05.

RESULTS

The whole-cell potassium currents (Fig. 1A) were recorded using the patch-clamp technique in the whole-cell configuration. The control values of the whole-cell conductance were greatly scattered with a mean of 4584 ± 865 pS (N = 20). To ascertain that the recorded currents were predominantly carried by potassium ions, their reversal potential was estimated by applying a standard approach based on a recording of the tail currents. Tail currents could be fitted by a single exponential, both under control conditions and in the presence of PR. Kinetics of tail currents was not significantly affected on PR treatment (data not shown). The reversal potentials in control conditions (−82.3 ± 2.4 mV, N = 6) and in the presence of 40 μM PR (−81.6 ± 3.1 mV, N = 6) were not significantly different ($P > 0.6$, Student's t-test). These values were close to the equilibrium potential for potassium ions (−87 mV) calculated from the Nernst equation (with intra- and extracellular K^+ concentrations as described in Materials and Methods). Under our experimental conditions, no inward currents were recorded at any depolarizing potential (see e.g. Fig. 1), indicating the lack of any significant contribution of voltage-dependent sodium and calcium currents. This is consistent with results obtained by other authors [12–21]. Because it is known that several types of Cl^- channels are present in human TL, it is important to assess whether or not the chloride component could contribute to the observed whole-cell currents. The calculated Nernst equilibrium potential for chloride ions is ca. −35 mV (with intra- and extracellular Cl^- concentrations as described in Materials and Methods). This value is far from the reversal potential estimated for the whole-cell currents under our experimental conditions. Results obtained by other authors confirm that the reversal potential of the TL whole-cell currents is close to the equilibrium potential for potassium ions, and is not influenced by intra- and extracellular chloride concentration [12–21]. Moreover, it was shown that, at room temperature, the activity of TL voltage-gated "maxi" Cl^- channels is strongly reduced in comparison to that measured at 37°C [31]. Activation of other types of TL chloride channels is unlikely under our experimental conditions [32, 33]. Altogether, the

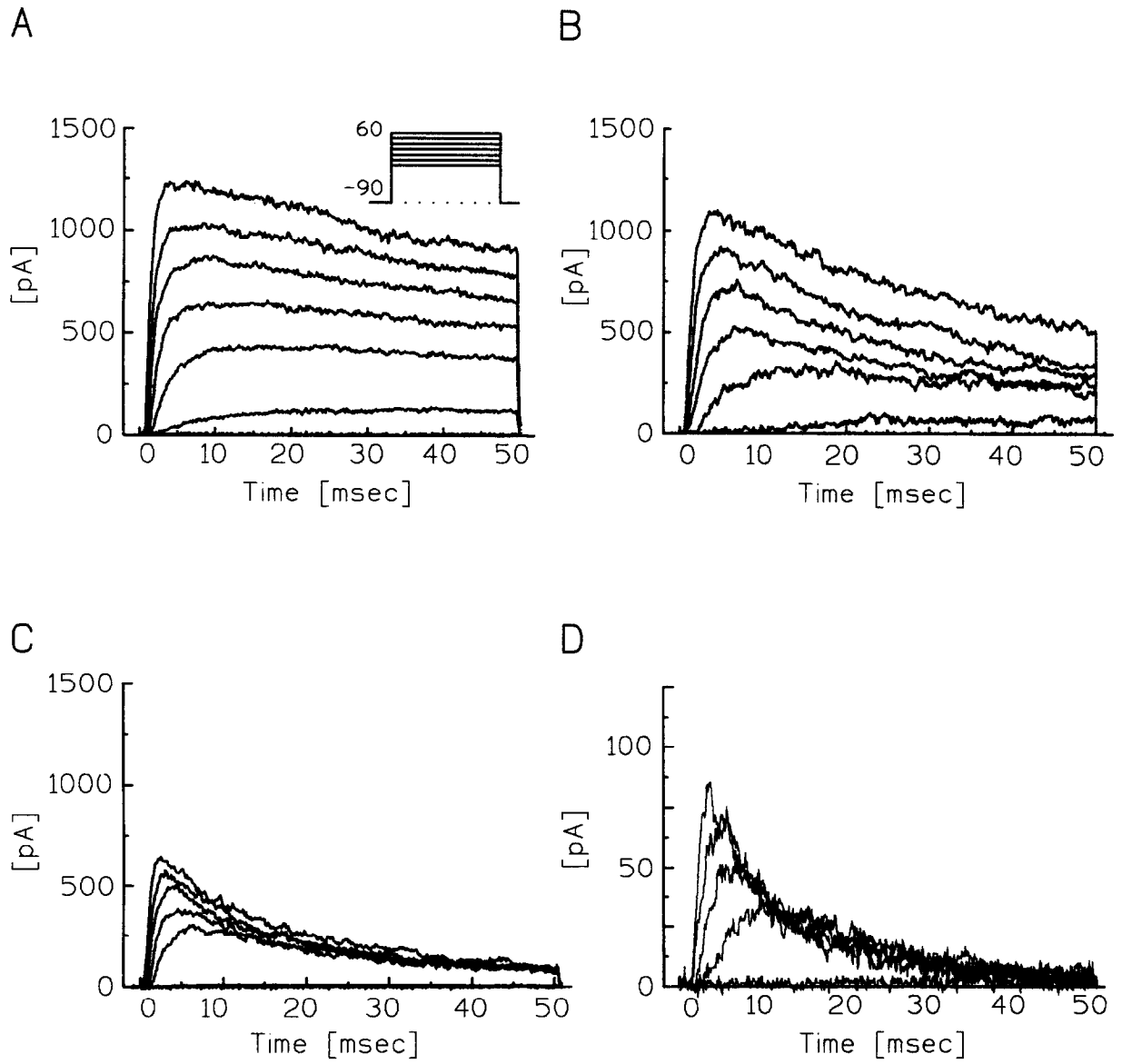

FIG. 1. Whole-cell potassium currents recorded from a human T lymphocyte under control conditions (A) and in the presence of PR at concentrations of: 20 (B), 40 (C), and 100 μM (D). Note expanded voltage scale in D. The depolarizing voltage protocol (seen schematically in the inset) is described in Materials and Methods.

data provide evidence that the whole-cell currents, both in control and in the PR-treated TL, were predominantly carried by potassium ions. The intracellular calcium concentration was kept low (ca. 100 nM) to prevent the activation of K_{Ca} channels in human TL [15]. Thus, the predominant component of whole-cell potassium currents recorded in our experiments was due to activation of K_V channels.

To study the effect of PR on the potassium currents in TL, the drug was gently added to the bathing saline surrounding the gigaseal patch, at different final concentrations. Results obtained under control conditions and at final PR concentrations of 20, 40, and 100 μM are depicted in Fig. 1. The addition of PR at a concentration of 20 μM caused a decrease in potassium currents (Fig. 1B). At this PR concentration, the relative decrease in current amplitudes was voltage-independent ($N = 5$, $F = 0.37$, $P > 0.05$, see Figs. 1B, 2). The current amplitudes were decreased to 83.1 ± 3.8% of the control values (averaged for all the voltages, $N = 20$). Application of PR at higher concentration (40 μM) caused a more pronounced decrease in potassium currents (see Fig. 1C). Also, for this PR concentration, relative decreases in current amplitudes were voltage-independent ($N = 5$, $F = 0.41$, $P > 0.05$, see also Figs. 1C, 2). Addition of 40 μM PR decreased the current amplitudes to approximately half of the control values (49 ± 1.3%, averaged for all the voltages, $N = 20$; see also Fig. 2). Further increase in PR concentration (100 μM) resulted in a voltage-independent ($N = 5$, $F = 0.43$, $P > 0.05$, see Figs. 1D, 2) decrease in potassium currents to 4.1 ± 0.69% of the control values (averaged for all the voltages, $N = 20$; see also Fig. 2). A complete block of the currents were observed when adding PR at concentrations in the range of 200–400

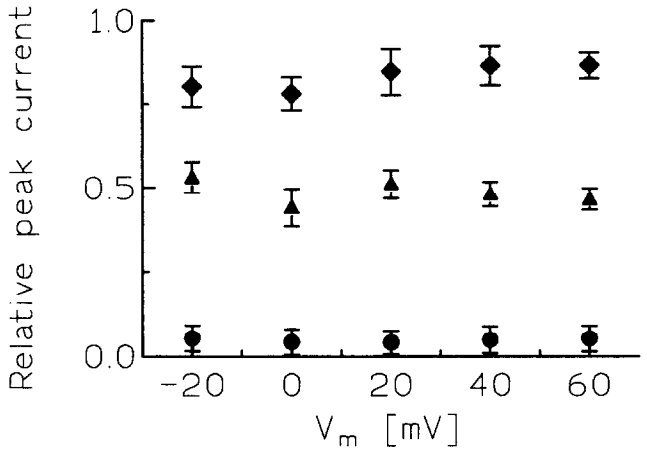

FIG. 2. Averaged relative peak current-to-voltage relationship. Relative peak current was obtained as a ratio of peak current recorded after addition of PR (20 μM, diamonds; 40 μM, triangles; 100 μM, circles) to the control peak current value recorded at the same membrane potential. Symbols and bars represent mean values ± SE (N = 5).

μM (data not shown). When washing out the drug with bathing solution, the currents were almost completely restored, indicating the reversibility of the block (N = 3, data not shown).

Figure 3 depicts typical examples of normalized whole-cell currents recorded at the same depolarizing potential under control conditions and after application of 20, 40, and 100 μM of PR. Apparently, the activation kinetics of the currents was not significantly affected upon PR treatment, whereas the inactivation process became faster and more profound (Fig. 3). The effect of PR on the inactivation phase of TL whole-cell potassium currents was clearly dose-dependent (Fig. 3).

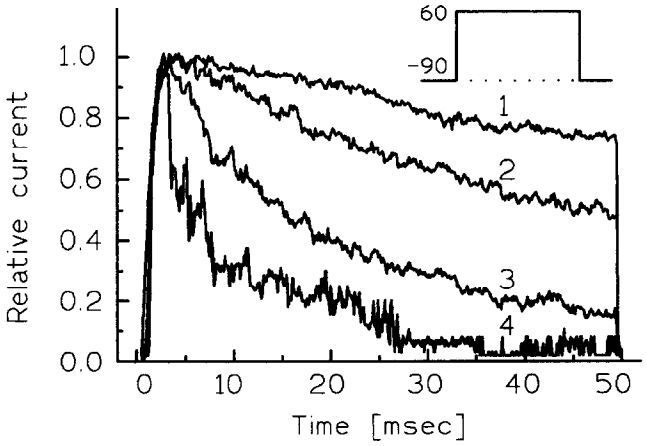

FIG. 3. Normalized currents obtained in response to application of a voltage step from -90 mV to +60 mV, under control conditions (trace 1) and in the presence of PR at final concentrations of: 20 (trace 2), 40 (trace 3), and 100 μM (trace 4). A strong dose-dependent effect of PR on the inactivation rate is seen, but the activation kinetics is not significantly affected.

To quantify the effect of PR on the activation and inactivation kinetics, the whole-cell currents were fitted applying Eqn (1). When fitting the control records with Eqn (1), thereby allowing the parameter p to assume real values (not only integer), the p parameter was in the range 4.31 ± 0.97 (SD), indicating that the best fit to the experimental data could be obtained with the integer value $p = 4$. The presence of PR did not change this pattern. Thus, both the control currents and those measured in the presence of PR were analyzed employing Eqn. (1), assuming $p = 4$. The activation time constants (τ_n), calculated in control and after application of 20 and 40 μM PR ($n = 10$), were voltage-dependent, showing a pronounced decrease with depolarization (Fig. 4). The values of τ_n in control and in the presence of 20 and 40 μM PR were not different at any depolarizing voltage applied (see Figs. 3, 4). Nor was any effect of propofol on the activation time constant observed at a higher drug concentration (100 μM-data not shown).

In our experiments, the decaying phase of the whole-cell currents could be well fitted with one exponential, both in control and in the presence of PR. Under control conditions, the inactivation time constant (τ_h) as well as the steady-state fraction of active channels (h) were voltage-dependent, showing a decrease with depolarization (Fig. 5A,B). In the presence of 20 μM PR, the τ_h value was decreased in comparison with control conditions (Figs. 3, 5A). The decrease was statistically insignificant (N = 5, $F = 1.92$, $P > 0.05$) probably because of the large disparity between the data (reflected by the large error bars overlapping one another; see also Fig. 5A). A significant decrease in the τ_h value was observed in the presence of 40 μM PR (N = 5, $F = 8.90$, $P < 0.05$; see also Figs. 3, 5A). Similarly, application of propofol at these final concentrations resulted in a pronounced decrease in the h parameter (Fig. 5B). In the presence of 20 μM PR, the decrease in h value was statistically insignificant (N = 5, $F = 2.09$, $P > 0.05$),

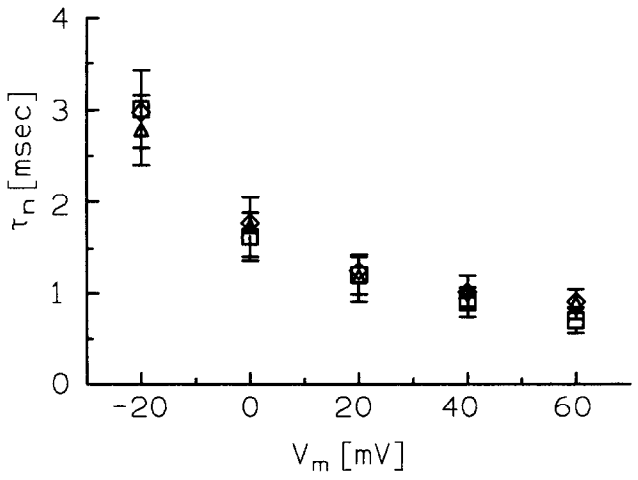

FIG. 4. Activation time constants (τ_n) calculated by applying Eqn (1) at different membrane potentials, under control conditions (squares), and in the presence of 20 (circles) and 40 μM PR (triangles). Symbols and bars represent mean values ± SE (N = 5).

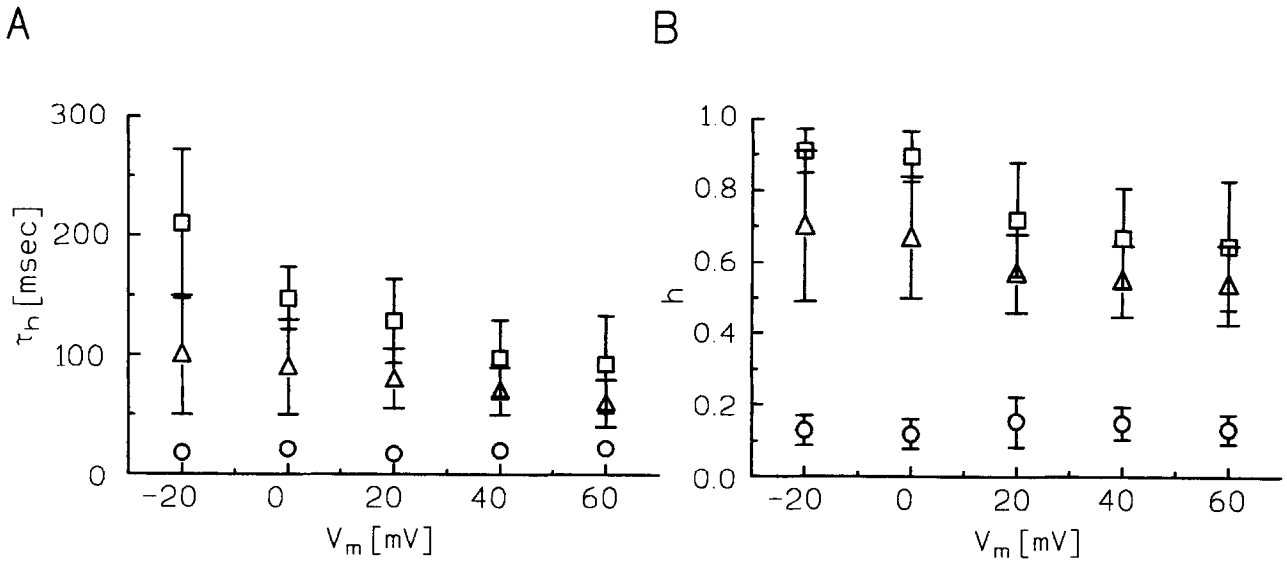

FIG. 5. (A) Inactivation time constants (τ_h) in control cells (squares), and in the presence of PR at final concentrations of 20 μM (triangles) and 40 μM (circles) vs membrane voltage. (B) Fraction of active channels (*h*) vs membrane voltage in control (squares), and after addition of PR at final concentrations of 20 μM (triangles) and 40 μM (circles). Symbols and bars represent mean values ± SE (N = 5). When smaller than the symbols, the error bars were omitted.

probably also due to the disparity between the data (Fig. 5B). The *h* value was significantly decreased in the presence of 40 μM PR (N = 5, $F = 25.51$, $P < 0.05$; see also Fig. 5B). Both parameters were further decreased when PR concentration was increased from 40 to 100 μM (data not shown). Thus, our data indicate that the effect of the drug on these parameters was dose-dependent. Moreover, in contrast to what was observed under control conditions, at PR concentration of 20 μM, the τ_h value was voltage-independent (N = 5, $F = 1.67$, $P > 0.05$; see also Fig. 5A). Furthermore, the *h* value was voltage-independent at this PR concentration (N = 5, $F = 1.19$, $P > 0.05$; see also Fig. 5B). Lack of voltage-dependence has also been observed at 40 μM PR concentration for both τ_h (N = 5, $F = 0.98$, $P > 0.05$; see also Fig. 5A) and *h* values (N = 5, $F = 1.01$, $P > 0.05$; see also Fig. 5B). A further increase in PR concentration up to 100 μM did not change this pattern (data not shown).

To ascertain that the observed blocking of K_V channels was due to the action of PR, a blank test with pure DMSO at concentrations of up to 1 mM was carried out; no effect on the whole-cell potassium currents was observed (data not shown).

DISCUSSION

Our data provide evidence that K_V channels in human TL are reversibly blocked by PR in a dose-dependent manner. A half-blocking concentration was approximately 40 μM. A dose-dependent increase in the rate and extent of inactivation together with the lack of any effect of PR on the activation time constant suggest an "open channel block" mechanism. Such blocking mechanism was proposed for voltage-gated potassium channels in squid giant axons internally dialyzed with solutions containing TEA or other quaternary ammonium ions [34, 35]. These authors showed that dialysis of giant axons with these solutions resulted in a dose-dependent decrease in peak current values and in the acceleration of the inactivation-like decay of potassium currents. An "open channel block" mechanism has also been proposed for WIN 17317-3 (1-benzyl-7-chloro-4-n-propylimino-1,4-dihydroquinoline hydrochloride), a novel inhibitor of K_V channels in human TL [36].

The presence of PR at concentrations of 20 and 40 μM resulted not only in a significant decrease of τ_h and *h* but also in an apparent loss of the voltage-dependence of these parameters (Fig. 5). Thus, because under control conditions τ_h and *h* values were voltage-dependent (but in the presence of 20 and 40 μM of PR were not), the relative decrease in both τ_h and *h* was also voltage-dependent.

The half-blocking PR concentration, of approximately 40 μM measured in our experiments lies within the same order of magnitude as those reported by other investigators for different types of channels in different cells. Frenkel and Urban [5] showed that human brain sodium channels are blocked by PR with IC_{50} of 20 μM. A significant reduction in calcium inward currents in guinea-pig isolated ventricular myocytes was observed by applying PR at concentrations in the range of 25–100 μM [7]. The open probability of voltage-dependent potassium channels in PC12 cells was reduced by 50 μM PR from 0.44 to 0.12 at 0 mV [9]. However, the block of K^+ channels in PC12 cells by PR was clearly voltage-dependent. Moreover, in these cells PR accelerated the activation of the whole-cell potassium currents and had no effect on the inactivation process. Similarly to what was observed in the present work, potassium channels in pig articular chondrocytes were blocked by PR in a voltage-independent manner, but the IC_{50} value was significantly lower (ca. 6 μM, [10]). Moreover, the kinetics

of ensemble-averaged potassium currents in pig articular chondrocytes was not affected by PR, indicating that, in this case, the mechanism of PR action was different from the "open channel block." Altogether, these facts indicate that the mechanisms of potassium channel block by PR in PC12 cells, pig articular chondrocytes, and human TL are different. On the other hand, because the voltage-dependent potassium channels in various cell types are known to be greatly diversified in their biophysical and pharmacological properties [37], such differences in PR-blocking mechanisms are not surprising.

The PR concentration that we found to reduce whole-cell potassium peak currents in human TLs by approximately 50% is of the same order of magnitude as blood PR concentrations employed for maintenance of general anesthesia (10–55 μM) [38]. However, it is known that the activity of voltage-gated potassium channels in human TL is required for cell physiological processes, such as mitogenesis and volume regulation. Available data provide evidence that blockers of voltage-gated potassium channels block also cell mitogenesis [23–27] and volume regulation in human TL [21, 28]. Therefore, the results of our study raise the question of the possible influence of PR on the TL cell functions in patients treated with this anesthetic. More studies, based on more than the electrophysiological approach, will be needed to further elucidate this problem. In particular, it would be interesting to investigate whether or not PR blocks TL mitogenesis at concentrations normally used in anesthesia.

In conclusion, our data provide further evidence that PR may block potassium channels in nonexcitable cells and suggest a need for investigations into the influence of PR on human TL cell functions.

The authors thank Dr. Sławomir Zmonarski for help in the separation and culture of the cells, Prof. Enrico Cherubini for valuable comments and suggestions and Gareth Owen for assistance in preparing the manuscript. This work was supported by the Polish Committee for Scientific Research (Komitet Badań Naukowych), Italian CNR, MURST, and University of Trieste.

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