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SPECIAL REPORT

Effect of descarboethoxyloratadine, the major metabolite of loratadine, on the human cardiac potassium channel Kv1.5

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The effects of descarboethoxyloratadine (DCL), the major metabolite of loratadine, were studied on a human cardiac K^+ channel (hKv1.5) cloned from human ventricle and stably expressed in a mouse cell line by means of the patch-clamp technique. DCL (1–100 μM) inhibited hKv1.5 current in a concentration-dependent manner with an apparent affinity constant of 12.5 ± 1.2 μM. The blockade increased steeply over the voltage range of channel opening, which indicated that DCL binds preferentially to the open state of the channel. At more depolarized potentials a weaker voltage-dependence was observed consistent with a binding reaction sensing $\approx 20\%$ of the transmembrane electrical field. DCL, 20 μM, increased the time constant of deactivation of tail currents, thus inducing a 'crossover' phenomenon. The present results demonstrated that DCL blocked hKv1.5 channels in a concentration-, voltage-, and time-dependent manner.

Keywords: hKv1.5 channels; Ltk^- cells; descarboethoxyloratadine; loratadine; histamine H_1 receptor antagonists; patch-clamp

Introduction Terfenadine and astemizole, two nonsedating histamine H_1-receptor antagonists, are potent blockers of K^+ channels, a property which has been considered to be responsible for QT prolongation of the electrocardiogram and life-treating ventricular tachycardias (torsades de pointes) (Woosley, 1996). Loratadine is a selective nonsedating histamine H_1-receptor antagonist prescribed for symptomatic relief of upper respiratory tract infections or allergy (Haria *et al*., 1994). Very recently, loratadine has been shown to produce both supraventricular and ventricular arrhythmias and sudden death (Lindquist & Edwards, 1997). Loratadine blocks open Kv1.5 channels cloned from human ventricle with a similar apparent affinity constant ($K_D = 1$ μM) (Delpón *et al*., 1997) to terfenadine (Yang *et al*., 1995). For astemizole, the parent compound and its principal metabolite, desmethylastemizole, block the delayed rectifier potassium current and, thus, both drugs may contribute to QT prolongation and the development of torsades de pointes (Vorperian *et al*., 1996). Loratadine is extensively metabolized to descarboethoxyloratadine (DCL), with peak plasma concentrations occurring within 1.5–3 h after dosing (Haria *et al*., 1994). Thus, it would be of great interest to examine whether DCL, the major active metabolite of loratadine, also blocks hKv1.5 channels.

Methods The experiments were performed at room temperature in mouse Ltk^- cells stably expressing hKv1.5 channels (Snyders *et al*., 1993). The hKv1.5 currents were recorded by use of the whole-cell configuration of the patch-clamp technique as previously described (Delpón *et al*., 1996; 1997; Valenzuela *et al*., 1996). Cells were perfused with an external solution containing (in mM): NaCl 130, KCl 4, $CaCl_2$ 1, $MgCl_2$ 1, HEPES 10 and glucose 10; pH was adjusted to 7.4 with NaOH. Recording pipettes were filled with an 'internal' solution containing (in mM): K-aspartate 80, KCl 42, KH_2PO_4 10, MgATP 5, phosphocreatine 3, HEPES 5 and EGTA 5 (adjusted to pH 7.2 with KOH). Results are expressed as mean ± s.e.mean. DCL was kindly provided by Almirall SA (Barcelona, Spain).

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Results Figure 1a shows superimposed hKv1.5 currents elicited by 500 ms pulses from −80 mV to different test potentials in control conditions and in the presence of 50 μM DCL. hKv1.5 currents were activated rapidly and then slowly and partially inactivated. Outward currents were followed by decaying outward tail currents upon repolarization to −40 mV. DCL reduced the peak current and induced an initial fast phase of decline of the current during the depolarizing pulse. In Figure 1b the current amplitudes at the end of 500 ms-pulses, in the absence and presence of 50 μM DCL, and values of the relative current ($I_{DCL}/I_{control}$) were plotted as a function of the membrane potential. In the right panel, the dotted line shows the activation curve in this particular experiment (midpoint and slope factor = −14.9 mV and 3.9 mV, respectively). DCL reduced the hKv1.5 current over the whole voltage range over which this current is activated. The blockade increased steeply between −30 mV and 0 mV (i.e. the voltage range for channel opening) and with a shallow voltage-dependence between 0 and +60 mV. Fitting these data following the Woodhull model the calculated fractional electrical distance (δ) was 0.17 ± 0.007 ($n = 9$).

Figure 2a shows the concentration-dependence of hKv1.5 inhibition with suppression of the current after 500 ms at +60 mV as an index of block. A nonlinear least-squares fit of the data yielded an apparent affinity constant (K_D) of 12.2 ± 1.5 μM and a Hill coefficient of 0.96 ± 0.1.

As shown in Figure 1a, DCL induced a falling phase in the current traces which was superimposed on the slow process of inactivation, so that current records positive to +40 mV were fitted to a biexponential function. The time constant of the fast falling phase was considered to represent the time constant of development of block (τ_{Block}), whereas the slow time constant reflects the slow and partial process of inactivation ($\tau \approx 260$ ms). In Figure 2b, the τ_{Block} was plotted as a function of DCL concentration and the experimental data were fitted to a hyperbolic function from which the apparent association (k) and dissociation rate constants (l) were calculated, averaging $(1.8 \pm 0.4) \times 10^6 M^{-1}s^{-1}$ and 22.4 ± 6.3 s^{-1} ($n = 15$), respectively.

The tail currents elicited on return to −40 mV after application of 500 ms depolarizing pulses to +60 mV reflected the irreversible closure of the channels at negative potentials and were fitted to a monoexponential function ($\tau = 31.3 \pm 4.4$ ms). DCL (20 μM) decreased the peak tail cur-

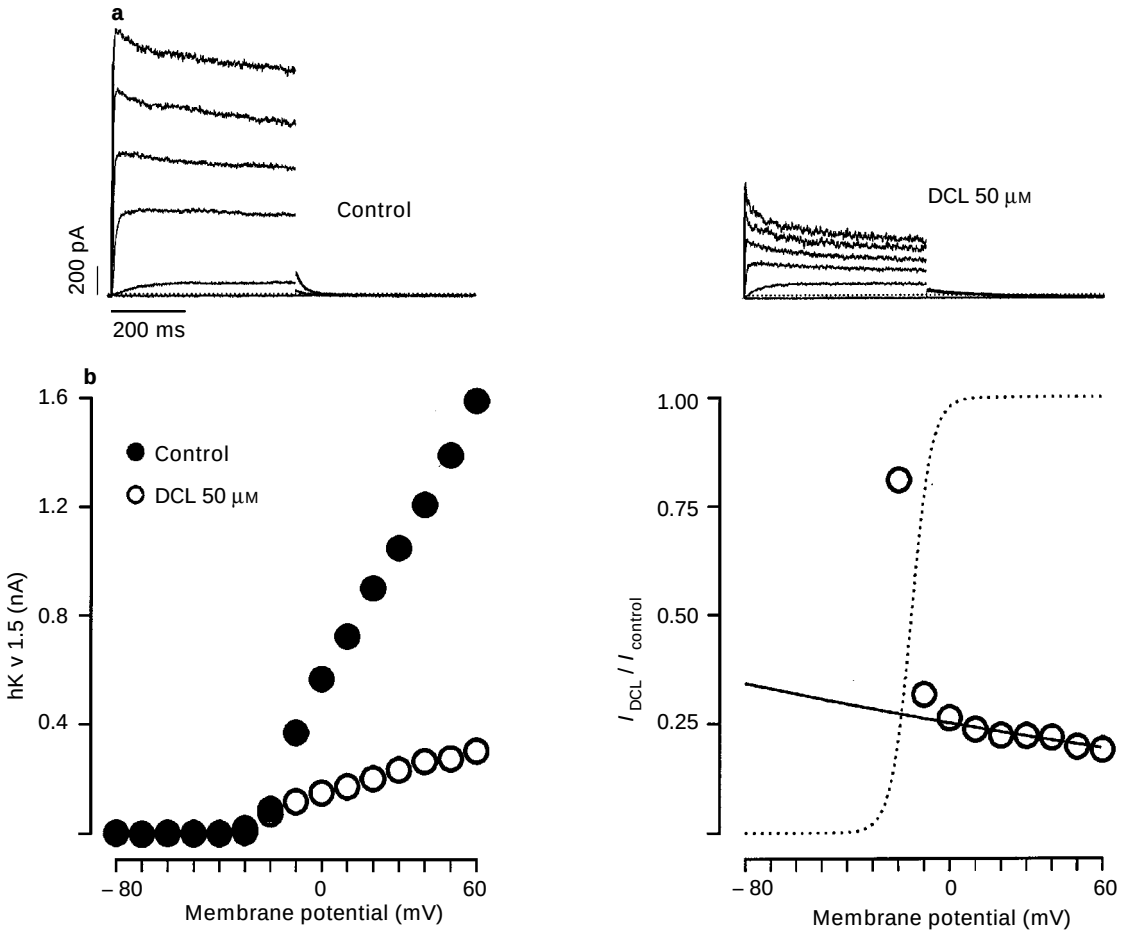

Figure 1 (a) Superimposed current traces are shown for 500 ms depolarizing pulses from −80 mV to voltages between −60 and +60 mV in steps of 20 mV in control conditions and in the presence of 50 μM DCL. (b) The left panel shows steady-state current-voltage (*I*-V) relationships (500 ms isochronal). The right panel shows the relative current expressed as $I_{DCL}/I_{control}$ from data shown on the left. The dotted line is the activation curve of the hKv1.5 channel for this particular experiment obtained by fitting the tail current amplitudes to the equation: $y = 1/[1 + \exp(V_h - V_m)/k]$, where V_h represents the midpotential of the curve, V_m the membrane potential and k the slope of the curve. Data of both panels were obtained from the same cell.

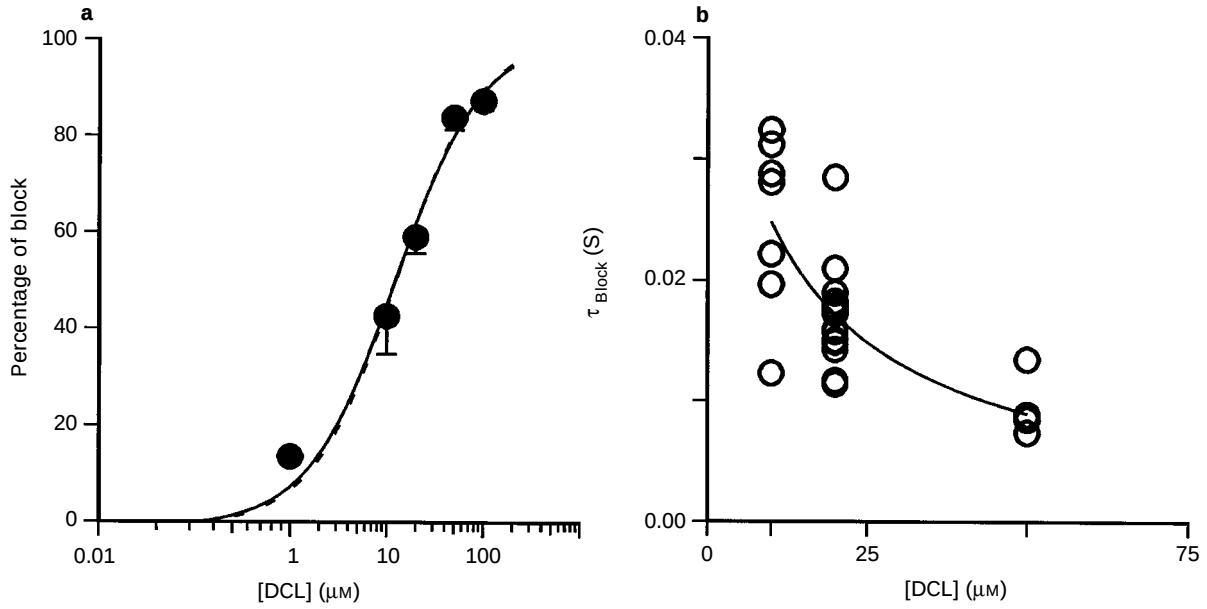

Figure 2 (a) Concentration-dependence of DCL-induced block of hKv1.5. The continuous line represents the fit of the experimental data to the equation $f = 1/\{1 + K_D/[D]^{n_H}\}$, where f is the fractional block, K_D is the apparent affinity constant, [D] is a drug concentration and n_H is the Hill coefficient. For comparison, the dashed line represents the fit of the experimental data for a Hill coefficient (n_H) of 1. Each point represents the mean and vertical lines show s.e.mean of 5–8 experiments. (b) The time constant of the fast DCL-induced component of decline of hKv1.5 current (τ_{Block}) was plotted versus DCL concentration. The solid line represents the fit to equation: $\tau_{Block} = 1/k \times [D] + l$), where k and l are the apparent association and dissociation rate constants, respectively.

rent amplitude and slowed the subsequent time course of the tails ($\tau = 102.9 \pm 13.3$ ms, $n = 5$; $P < 0.01$), producing a crossover phenomenon of the tail currents.

Discussion The present results demonstrated tht DCL inhibits hKv1.5 channels which generated the ultrarapid delayed outward K^+ current in human atria (Wang *et al*., 1993). DCL induced a concentration-dependent block of hKv1.5 channels, but was less potent than loratadine ($K_D = 1$ μM; Delpón *et al*., 1997) and terfenadine ($K_D = 0.8$ μM) (Yang *et al*., 1995). The K_D value for DCL was about 100 times higher than the peak plasma concentrations (28 ng ml^{-1}) found in healthy volunteers (Haria *et al*., 1994). However, it is difficult to relate the *in vivo* plasma concentrations to those of drug-perfused isolated cells, particularly when the drug is highly bound to plasma and tissue proteins. The blockade increased steeply in the voltage range of channel activation, which suggests that this drug binds preferentially to the open state of the channel. Since DCL ($pK_a = 8.9$) predominates in the cationic form at the intracellular pH of 7.2, the voltage-dependence at potentials positive to 0 mV can be attributed to the effect of the transmembrane electrical field on the interaction between the cationic molecule and the channel receptor. The δ value indicated binding at a site $\approx 20\%$ into the transmembrane electrical field as referenced from the intracellular side. The k value obtained for DCL was twice as slow, while the l value appeared to be 6 times faster than that of loratadine. This explains why DCL was less potent than the parent compound. Furthermore, DCL induced a crossover of the tail currents, which indicated that drug unbinding is required before channels can close and provided further evidence for the proposed open channel interaction.

Block of hKv1.5 channels may explain the supraventricular arrhythmias obtained with loratadine as well as some ventricular arrhythmias which are preceded by rapid supraventricular rhythms. Further studies evaluating the effects of loratadine and DCL on other human cardiac K^+ channels (e.g. HERG, I_{Ks}) are needed to confirm the clinical risks of these drugs.

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