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Effects of azelastine on contractility, action potentials and L-type Ca^{2+} current in guinea pig cardiac preparations

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Abstract

Azelastine is used for symptomatic relief of allergic rhinitis and asthma bronchiale. In vitro studies in smooth muscle cells from guinea pig trachea and ileum demonstrate that the drug blocks L-type Ca^{2+} current ($I_{Ca,\,L}$). However, for safety reasons, it is important to know whether azelastine also affects cardiac $I_{Ca,\,L}$ in therapeutically relevant concentrations. We have therefore studied the effects of azelastine on $I_{Ca,\,L}$ in guinea pig ventricular myocytes using standard whole-cell patch-clamp technique. Force of contraction and action potentials from isolated papillary muscles of the same species were also investigated at physiological temperature (36°C). Azelastine (30 μM) significantly reduced force of contraction, shortened action potential duration, and depressed maximum upstroke velocity. $I_{Ca,\,L}$ was elicited by 200-ms-long clamp steps from −100 to 0 mV (one pulse every 3 s). Azelastine blocked $I_{Ca,\,L}$ reversibly and concentration-dependently with an IC_{50} of 20.2 ± 1.3 μM and a Hill coefficient of 1.1. At 10 μM, azelastine shifted steady-state inactivation by 5 mV ($n = 7$) to more negative potentials. The time course of $I_{Ca,\,L}$ inactivation could be described by a double exponential function. Azelastine (10 μM) significantly shortened the slow inactivation time constant (τ_s) from 54.2 ± 2.8 ms under control conditions to 38.7 ± 2.9 ms ($n = 16$) in the presence of drug. Azelastine also reduced low-voltage-activated Ca^{2+} currents with a similar IC_{50} value (24 μM, at −35 mV). Since the therapeutic plasma concentrations are in the order of 10–100 nM, we conclude that azelastine does indeed affect also cardiac $I_{Ca,\,L}$, but the concentrations required are at least two orders of magnitude larger than those obtained during drug therapy.

Keywords: Azelastine; Action potential; Ca^{2+} current, L-type; Ca^{2+} channel; Na^{+} channel; Papillary muscle; Myocyte

1. Introduction

The second-generation histamine H_1 receptor antagonist azelastine [4-(*p*-chlorobenzyl)-2-(hexahydro-1-methyl-1*H*-azepin-4-yl)-1-(2*H*)-phthalazinone hydrochloride] is currently used as an anti-asthmatic and anti-allergic drug. One component of the anti-asthmatic action of azelastine was suggested to be related to its profound effects on intracellular Ca^{2+} handling. Azelastine decreased basal intracellular calcium concentration ($[Ca^{2+}]_i$) in cultured rabbit airway smooth muscle cells ($IC_{50} = 110$ μM), and impaired the endothelin-1-induced increase in $[Ca^{2+}]_i$ by inhibiting intracellular Ca^{2+} mobilisation ($IC_{50} = 67$ μM; Senn et al., 1991). The platelet-activating factor-induced increase of $[Ca^{2+}]_i$ was reduced by azelastine in guinea pig peritoneal macrophages ($IC_{50} = 16$ μM; Nakamura et al., 1988), and at lower concentrations in the eosinophilic leukaemia cell line EoL-1 ($IC_{50} = 11$ nM; Morita et al., 1993). In addition, azelastine was shown to inhibit histamine release by interfering with Ca^{2+} influx in rat peritoneal mast cells with an IC_{50} value of 5 μM (Chand et al., 1983). A direct inhibition of ion currents was shown in smooth muscle cells isolated from guinea pig trachea. Ba^{2+} currents conducted by L-type Ca^{2+} channels were completely suppressed by azelastine with an IC_{50} value of 8 μM (Hazama et al., 1994). Furthermore, the compound reduced nifedipine-sensitive Ca^{2+} inward currents in isolated myocytes from guinea pig ileum ($IC_{50} = 13$ μM; Masuo et al., 1992).

Little is known about the effects of azelastine on ionic currents in heart muscle despite the fact that impairment of Ca^{2+} and/or K^{+} currents may compromise excitation–contraction coupling and alter the shape of the cardiac action potential. Azelastine at concentrations from 10 to 100 μM has been studied in partially depolarized guinea pig papillary muscles, where the drug depressed Ca^{2+}-de-

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pendent slow action potentials and accompanying contractions induced by isoproterenol in high-K^+ solution (Molyvdas et al., 1989; Li and Zhang, 1992). This indirect approach provides evidence for an inhibitory action of azelastine on cardiac Ca^{2+} channels. However, the same drug concentrations also markedly reduced the maximum upstroke velocity (dV/dt_{max}) at normal extracellular K^+ concentrations, where the tissues were not depolarized, indicating block of Na^+ current (Molyvdas et al., 1989).

Ca^{2+} channel blockers are well known to reduce cardiac force of contraction (F_c) and produce negative chronotropic and dromotropic effects, and hence may aggravate cardiac dysfunction in certain patients at risk. We could confirm a negative inotropic and an action potential duration shortening effect of high concentrations of azelastine (30 μM). The aim of the present study was to investigate whether azelastine affects cardiac L-type Ca^{2+} current in order to assess the putative risk for drug-induced cardiac side effects based on modulation of these currents. We found that the drug does in fact block both low-voltage-activated Ca^{2+} currents (LVACC, i.e. T-type and tetrodotoxin-sensitive Ca^{2+} currents; Heubach et al., 2000), and high-voltage-activated current (HVACC, i.e. $I_{Ca,\ L}$) albeit at high concentrations only.

2. Materials and methods

2.1. Action potentials

Right ventricular papillary muscles from the hearts of male guinea pigs (200–350 g) were mounted in a small muscle chamber and were continuously superfused with solution A (2 ml/min, composition see below) at 36°C. One end of the muscle was pinned to the floor of the chamber; the free end was connected to a force transducer (AE 801, SensoNor, Dasing, Germany) with a loop of silk thread. The muscles were allowed to equilibrate for 60 min at a stimulation frequency of 1 Hz, before action potentials were recorded with conventional microelectrodes of 14–50 MΩ tip resistance when filled with 2.5 M KCl. F_c was displayed on an oscilloscope and recorded digitally on a personal computer. Since drug effects may be rate-dependent, four different rates of stimulations were studied. After the equilibration period, the pre-drug control values at 0.1, 0.5, 1 and 2 Hz were determined, before stimulation frequency was set to 1 Hz again and preparations were exposed to azelastine (30 μM). The action potential parameters were continuously recorded, and analysed at regular intervals. Drug effects were also tested at 0.1, 0.5 and 2 Hz 45 min after drug addition and were compared with the respective frequency response before addition of drug. For this purpose, stimulation was stopped for 90 s prior to change of frequency. Steady state of the action potential was reached after 3 min of regular stimulation at each frequency. The recorded signals were amplified (KS-700, WPI, Berlin, Germany), displayed on the oscilloscope and also stored on-line. All data acquisition and analysis were carried out with an ISO-2 system (MFK, Niedernhausen, Germany).

2.2. Cell dissociation

The enzymatic isolation procedure has been described previously (Heubach et al., 2000). Briefly, male guinea pigs of 250–350 g body weight were anaesthetized with 70% CO_2 and 30% O_2 prior to decapitation. The excised hearts were mounted on a Langendorff apparatus for perfusion of the coronary arteries. Blood was removed by a 3-min period of perfusion with oxygenated solution B, which was followed by 5 min of perfusion with nominally Ca^{2+}-free solution B. Enzymatic digestion was initiated by 2 min of perfusion with collagenase-containing buffer and further 2 min of washing in enzyme-free buffer. The ventricles were then cut off, chopped into small chunks and stirred in a small temperature-regulated vessel containing storage solution until elongated, striated myocytes dissociated from the tissue pieces. Myocytes were harvested by filtering the cell-containing suspension through a nylon filter (200 μm). They were washed three times in storage solution and then maintained at room temperature in the same buffer until used for experimentation.

2.3. Patch-clamp recordings

A small drop of cell suspension was transferred to a chamber situated on the stage of an inverted microscope. The chamber was continuously perfused at a rate of 1.6 ml/min with the cell superfusion solution. The bath temperature was maintained at 36 ± 0.5°C.

Currents were measured in the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) with a patch-clamp amplifier EPC-7 (List Electronics, Darmstadt, Germany). Data of currents were acquired and analysed with the software pClamp 5.5 and 6.0, respectively. Glass pipettes had tip resistances of 2.5–5 MΩ when filled with pipette solution (see below). After seal formation and disruption of the membrane, cell capacitance was measured by a short hyperpolarising ramp pulse from a holding potential of −40 mV (5 mV in 5 ms). Since membrane conductance is very low and constant in this range, any change in current level is due to the capacitive properties of the cell membrane (Amos et al., 1996). The average capacitance was 146.5 ± 7.5 pF ($n = 38$). Up to 100 pF of the cell capacitance and about 50% of the access resistance was then electronically compensated. Leak currents and liquid junction potentials were not corrected.

Voltage-activated Ca^{2+} currents were evoked by 200-ms test pulses to 0 mV every 3 s from a holding potential of −100 mV. Contaminating K^+ currents were eliminated by K^+ substitution both in the pipette and the superfusion

solutions, and Na^+ current was eliminated by substituting extracellular NaCl with tetraethylammonium chloride. Current–voltage relationship was obtained by applying pulses for 200 ms between −60 and 80 mV in increments of 5 mV from a holding potential of −100 mV. Activation curves were obtained by dividing current amplitude by driving force, $G = I/(V_m - E_{rev})$, where G and I are Ca^{2+} conductance and current, respectively, at test potential V_m, and E_{rev} is the reversal potential assumed as 70 mV (Fig. 3A). Steady-state inactivation curves were obtained by applying test pulses for 200 ms from −80 to 0 mV, 5 ms after 2-s conditioning pulses ranging from −60 to +20 mV and were fitted with the Boltzmann function: $I/I_{max} = 1/(1 + \exp((V_m - V_{0.5})/K))$, where $V_{0.5}$ and K are the half-maximum inactivation potential and the slope factor, respectively. The time constants for the inactivation process were obtained by fitting a double exponential function to individual current traces: $I(t) = A_f(1 - \exp(-t/\tau_f)) + A_s(1 - \exp(-t/\tau_s))$, where A_f and τ_f are the amplitude and inactivation time constant of the fast-inactivating component, and A_s and τ_s are the amplitude and inactivation time constant of the slow-inactivating component, respectively.

Concentration–response curves were fitted using the non-linear regression $Y = 1/(1 + 10^{(\log IC_{50} - X)n_H})$, where X is the decadic logarithm of the concentration used, IC_{50} is the concentration at which the half-maximum effect occurs, and n_H is the Hill coefficient.

2.4. Solutions

For action potential measurements, a modified Tyrode's solution (solution A) of the following composition was used (in mM): NaCl 126.7, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 22, NaH_2PO_4 0.42, glucose 5.6. The pH was adjusted to 7.4 by bubbling the action potential Tyrode with a mixture of 95% O_2 and 5% CO_2. The solution for myocyte isolation (solution B) was composed of (in mM): NaCl 150, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 2, HEPES 10, glucose 11; pH 7.4. Nominally Ca^{2+}-free solution was

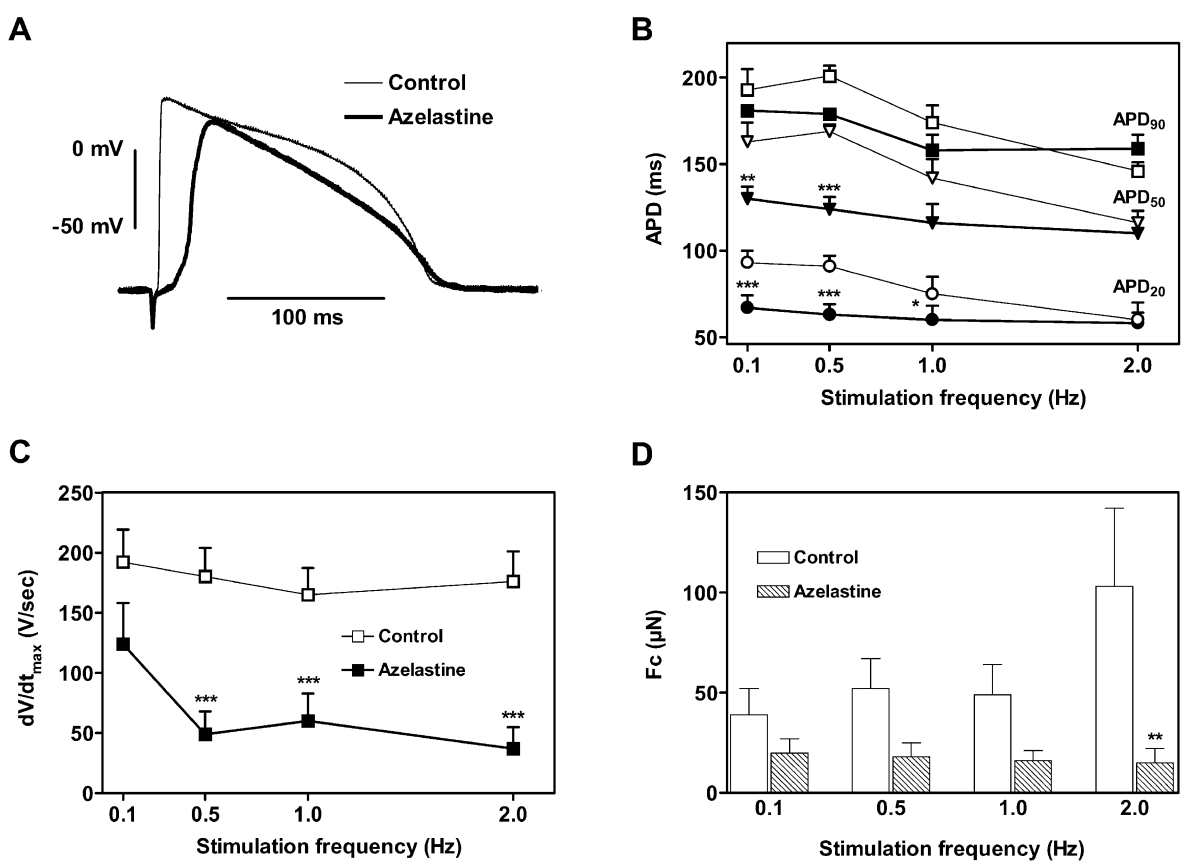

Fig. 1. Effects of azelastine (30 μM) on the action potential and F_c of guinea pig papillary muscle. (A) Original traces of action potential in the absence (control) and presence of 30 μM azelastine at 1 Hz. (B) Frequency dependence of action potential duration at 90%, 50% and 20% repolarization (APD_{90}, APD_{50} and APD_{20}, respectively, open symbols) and effects of azelastine (30 μM, closed symbols). (C) Frequency dependence of dV/dt_{max} under control conditions (open symbols) and in the presence of azelastine (30 μM, closed symbols). (D) Effect of azelastine on F_c of papillary muscles (control F_c, white columns; in the presence of azelastine, black columns). Mean values ± S.E.M. from $n = 5$ experiments. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control (repeated-measures analysis of variance followed by Bonferroni post-test).

the same solution albeit with $CaCl_2$ omitted. Enzyme-containing solution consisted of nominally Ca^{2+}-free solution complemented with 0.05% (w/v) collagenase type 1, 0.003% (w/v) pronase and 0.8 mg/ml albumin. The buffer for cell storage was composed of (in mM): KCl 40, KOH 70, L-glutamic acid 50, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, HEPES 10, EGTA 0.5, glucose 10; pH 7.4. The solution for cell superfusion contained (in mM): tetraethylammonium chloride 120, HEPES 10, $MgCl_2$ 1, CsCl 10, glucose 10, $CaCl_2$ 2; pH 7.4. The pipette-filling solution was composed of (in mM): CsCl 20, Cs-methane-sulfonate 90, EGTA 10, HEPES 10, Mg-ATP 4, Tris-GTP 0.4, $CaCl_2$ 3; the pH was adjusted to 7.2 with CsOH.

2.5. Drugs

Azelastine chloride as well as the D- and L-enantiomers were kindly provided by Arzneimittelwerk Dresden. Stock solution (10 mM) of azelastine in distilled water was prepared and stored at −20°C until use.

2.6. Statistics

All data are expressed as means ± S.E.M. Differences between groups were analysed statistically by repeated-measures analysis of variance followed by Bonferroni post-test, or by Student's *t*-test. Levels of significance are $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

3. Results

3.1. Effects of azelastine on the action potentials of guinea pig ventricular papillary muscles

In a first series of experiments, we tested the effects of azelastine on action potentials and F_c (Fig. 1). A high concentration (30 μM) was chosen in order to obtain a near-maximum response. Within 45 min of exposure to the drug, the dV/dt_{max} and the amplitude of the action potentials were markedly reduced, whereas the resting potential was not affected (Fig. 1A). The action potential duration (APD) was markedly shortened at levels of 20% and 50% repolarisation (Fig. 1A,B). The effects of azelastine on APD depended on the frequency of stimulation (Fig. 1B). At 0.1 Hz, APD_{90} decreased from 193 ± 12 to 181 ± 3 ms, APD_{50} from 163 ± 11 to 130 ± 7 ms and APD_{20} from 93 ± 7 to 67 ± 7 ms ($n = 5$); the respective values at 0.5 Hz were: APD_{90} from 201 ± 6 to 179 ± 2 ms, APD_{50} from 169 ± 4 to 124 ± 7 ms and APD_{20} from 91 ± 6 to 63 ± 6 ms ($n = 5$). At frequencies ≥ 1 Hz, however, the changes did not reach the level of statistical significance. The values of dV/dt_{max} were significantly depressed by 30 μM azelastine at all frequencies with exception of 0.1 Hz (Fig. 1C). F_c was significantly reduced by 30 μM azelastine at 2 Hz (Fig. 1D).

3.2. Effects of azelastine on Ca^{2+} currents in guinea pig ventricular myocytes

Typical L-type Ca^{2+} currents ($I_{Ca,\,L}$) elicited by test pulses to 0 mV from a holding potential of −100 mV (Fig. 2) had an average peak value of −9.0 ± 0.7 pA/pF ($n = 38$). This current was completely and reversibly blocked by 100 μM Cd^{2+} ($n = 4$, data not shown). Exposure to azelastine started 5 min after rupture of the membrane and the effects were recorded 5 min after exposure. During this period, the run-down of $I_{Ca,\,L}$ amounted to 17 ± 7% ($n = 12$) within time-matched control myocytes. Azelastine (30 μM) markedly reduced $I_{Ca,\,L}$ and the effect was partially reversible during removal of the drug (Fig. 2A). The complete concentration–response curve for the inhibitory effect of azelastine applied in a non-cumulative manner (Fig. 2B) yielded an IC_{50} of 20.2 ± 1.3 μM and a Hill coefficient of 1.1. The inhibitory effect of azelastine on $I_{Ca,\,L}$ became statistically significant at 10 μM ($P < 0.001$).

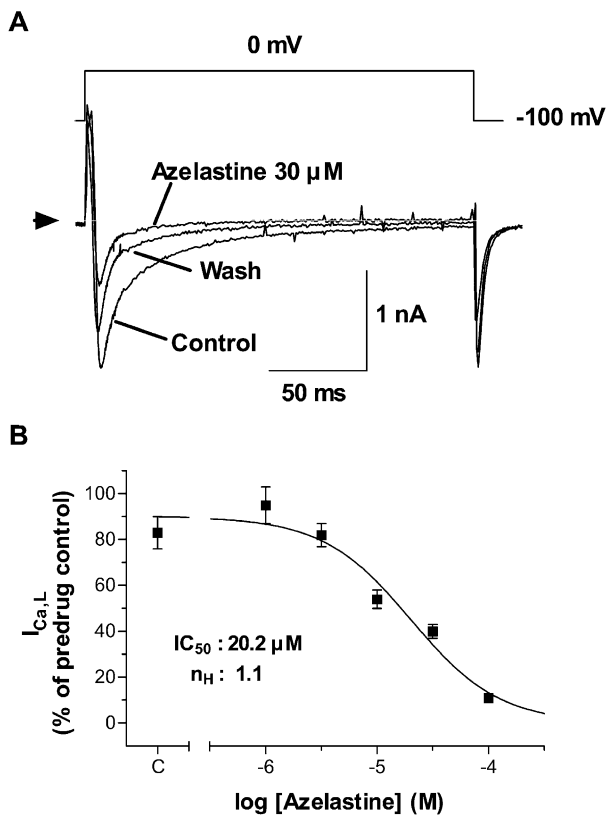

Fig. 2. Effect of azelastine on L-type Ca^{2+} current $I_{Ca,\,L}$ of guinea pig ventricular myocytes. (A) Original current traces in response to 200-ms-long clamp pulses to 0 mV from a holding potential of −100 mV before, 5 min after addition of azelastine (30 μM) and after 10 min of drug wash-out. The arrowhead points at 0 current level; scale bars as indicated. (B) Concentration–response curve for the effect of azelastine on $I_{Ca,\,L}$. Currents are normalised to pre-drug control values and expressed as means ± S.E.M. from $n = 3$–12 myocytes. A single concentration was tested in each cell. Run-down of $I_{Ca,\,L}$ amounted to 17 ± 7% in time-matched control myocytes (C; $n = 12$).

The current–voltage relationship for Ca^{2+} currents between −60 and 80 mV (in increments of 5 mV, holding potential of −100 mV) revealed the presence of both low-voltage and high-voltage-activated currents (LVACC, and HVACC that corresponds to $I_{Ca,\,L}$, respectively; Fig. 3A). Azelastine concentration-dependently reduced both current components. When the drug effect was analysed at −35 mV (potential of peak LVACC), the IC_{50} value for the azelastine-induced block of LVACC was 24 μM.

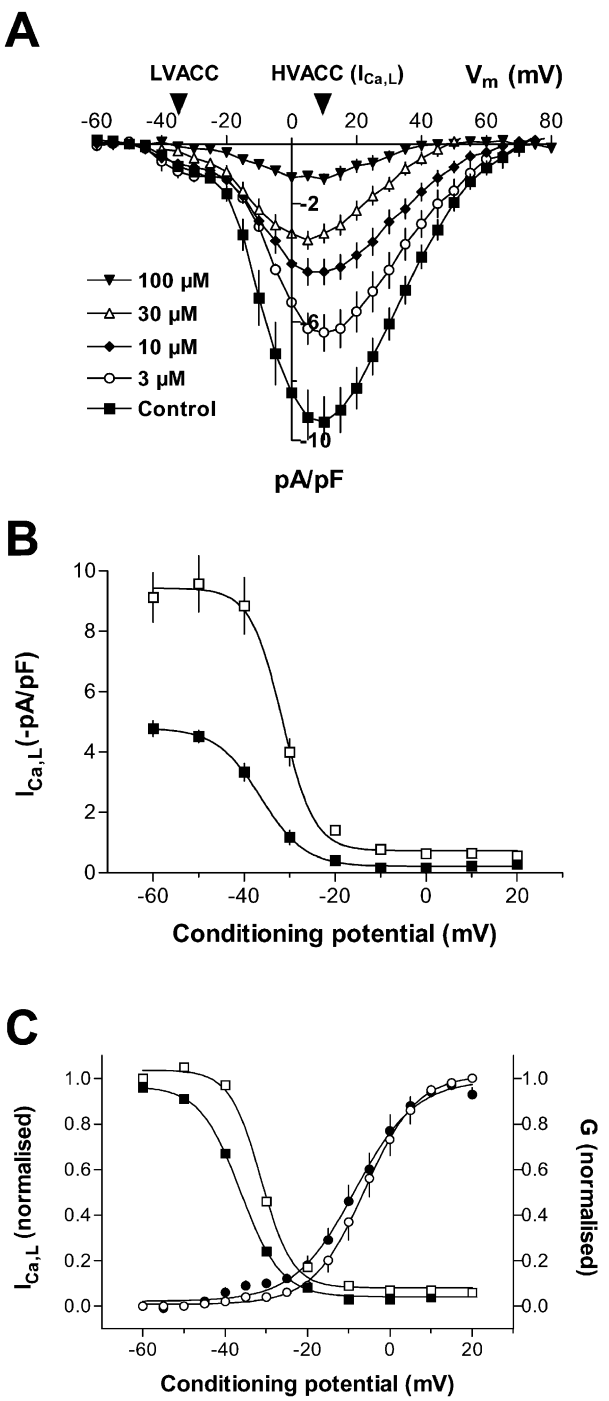

Fig. 3. Effects of azelastine at different concentrations on current–voltage (I–V) relation, activation and inactivation of $I_{Ca,\,L}$ in guinea pig ventricular myocytes. Cells were exposed to azelastine at about the 5th minute from the establishment of the whole-cell configuration and the current curves were obtained at about the 15th minute. For steady-state inactivation, test pulses were applied for 200 ms from −80 to 0 mV 5 ms after 2-s conditioning pulses ranging from −60 to +20 mV. (A) I–V curves obtained from peak current amplitudes; 200-ms steps from a holding potential of −100 mV were applied between −60 and 80 mV (increments of 5 mV). (Control) Pre-drug control for all cells ($n = 28$) exposed to the various concentrations ($n = 4$–11). Note that appreciable current is activated at potentials below the threshold for $I_{Ca,\,L}$, i.e. at −40 to −30 mV (low-voltage-activated Ca^{2+} current, LVACC). Effects of azelastine (10 μM) on the steady-state inactivation curves, with absolute (B) and normalised (C) mean values from seven cells measured either without drug (control, open squares) or in the presence of drug (closed squares). (C) demonstrates normalized activation curves of $I_{Ca,\,L}$ in the absence (open circles) and presence (closed circles) of 10 μM azelastine.

From the I–V curves, we calculated $I_{Ca,\,L}$ activation curves by correcting current amplitude at individual voltages for the driving force assuming a reversal potential E_{rev} of 70 mV under our experimental conditions (Fig. 3A). Although azelastine clearly reduced $I_{Ca,\,L}$ amplitude, it did not significantly shift the voltage dependence of the activation curve (Fig. 3C). The $V_{0.5}$ was -5.7 ± 1.9 mV before drug exposure and -7.7 ± 1.9 mV ($n = 9$) in the presence of 10 μM azelastine. The steady-state inactivation curves were obtained with test pulses to 0 mV after 2-s conditioning pulses ranging from −80 to +40 mV before and after exposure to azelastine (Fig. 3B,C). Azelastine (10 μM) significantly shifted the inactivation curve to more negative potentials: $V_{0.5}$ was -31.4 ± 0.6 mV under control condition and -36.2 ± 0.2 mV ($n = 7$, $P < 0.001$) in the presence of azelastine.

The time course of $I_{Ca,\,L}$ inactivation could be described by two exponential functions. Azelastine (10 μM) significantly increased the speed of the slower inactivation component. The time constant τ_s was shortened from 54.2 ± 2.8 ms under pre-drug control conditions to 38.7 ± 2.9 ms in the presence of azelastine ($n = 16$, $P < 0.01$; Fig. 4). The time constant τ_f of the fast-inactivating component was not significantly affected; the respective values were 2.8 ± 0.3 ms before and 3.0 ± 0.2 ms after addition of azelastine.

In order to gain insight into the nature of the block produced by azelastine, we investigated whether the effect was voltage-dependent as for instance observed with dihydropyridine derivatives (Porzig, 1990). To this purpose, we have also measured the blocking effect of azelastine on $I_{Ca,\,L}$ at a more depolarised holding potential (−60 mV). Azelastine (30 μM) reduced $I_{Ca,\,L}$ to the same extent at the two holding potentials, i.e. to $45 \pm 11\%$ at −60 mV and to $40 \pm 3\%$ at −100 mV ($n = 3$ and 10, respectively).

Since azelastine is the racemic mixture of two stereoisomers, the blocking effect could reside in only one of the

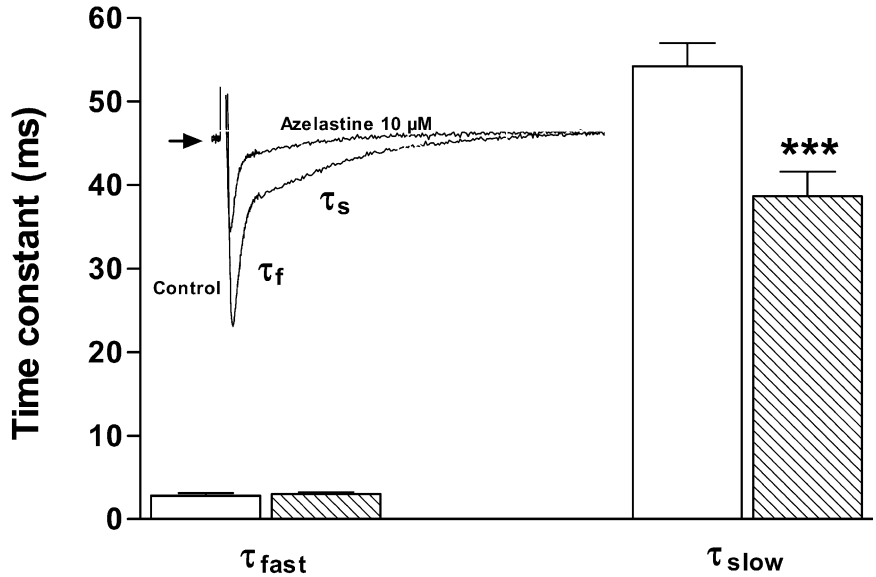

Fig. 4. Time course of current inactivation. Data were fitted with a double exponential function; τ_f: fast time constant of inactivation; τ_s: slow time constant of inactivation; $^{***}P < 0.001$ vs. pre-drug control (Student's *t*-test).

enantiomers. We have therefore studied the D- and L-enantiomers separately. At 15 μM, both enantiomers blocked $I_{Ca,\,L}$ to a similar degree, i.e. with D-azelastine to 40 ± 8% ($n = 5$) and L-azelastine to 39 ± 5% ($n = 5$).

4. Discussion

From previous reports in the literature, azelastine appears to possess a wide spectrum of pharmacological actions (McTavish and Sorkin, 1989), most of which were associated with a decrease in $[Ca^{2+}]_i$ (Nakamura et al., 1988; Senn et al., 1991; Masuo et al., 1992; Hazama et al., 1994). The main new findings of our work are that azelastine changes the shape of the cardiac action potential in a frequency-dependent manner and that it reduces Ca^{2+} currents in guinea pig ventricular myocytes albeit at concentrations more than 100-fold higher than the therapeutic plasma concentration. Possible relations between these two effects are discussed below.

4.1. Effects of azelastine on action potentials

Azelastine profoundly diminished action potential amplitude and rate of depolarisation during the upstroke (dV/dt_{max}), and reduced the duration of the plateau phase (APD_{20} and APD_{50}). Despite decreased values for APD_{90} in the presence of azelastine, the time between stimulus artifact and final repolarisation was not changed due to the large delay during the upstroke (compare Fig. 1A). The drug's effect on amplitude and dV/dt_{max} suggest that Na^+ current is blocked, which is in line with previous reports (Molyvdas et al., 1989). Moreover, the depression was significant at all but the lowest stimulation frequency tested suggesting that the effect shows little use-dependence. The shortening of the plateau phase can be explained by an inhibitory effect on L-type Ca^{2+} current (see below). Interestingly, the effect was more pronounced at 0.1 and 0.5 Hz, while it no longer reached the level of statistical significance at 2 Hz. This finding could be interpreted as "reversed" use-dependence, i.e. less effectiveness at higher rates of stimulation. However, since the shape of the action potential is determined by the sum of all inward and outward currents, it cannot be excluded that additional drug effects on other currents may counteract the plateau shortening produced by block of $I_{Ca,\,L}$.

Azelastine also significantly depressed F_c at a stimulation frequency of 2 Hz and tended to reduce contractility at all lower frequencies investigated. Guinea pig ventricle depends on both Ca^{2+} influx and Ca^{2+} release from the sarcoplasmic reticulum for contractile activation (Barry and Bridge, 1993; Bluhm et al., 1999); therefore, the negative inotropic effect of azelastine may be related to effects on both processes: sarcoplasmic reticulum Ca^{2+} stores are probably filled to a lesser extent because of Ca^{2+} influx inhibition in the presence of azelastine, resulting in a decrease of force at all frequencies studied (Fig. 1D). All these results provide an indirect evidence for azelastine's inhibition of Ca^{2+} channels and are in line with the block of L-type Ca^{2+} current reported in guinea pig ileum smooth muscle (Masuo et al., 1992). Furthermore, azelastine has been shown to inhibit contractility of cardiac papillary muscle and ileum and airway smooth muscles (Molyvdas et al., 1989; Masuo et al., 1992; Senn et al., 1991).

4.2. Effects of azelastine on Ca^{2+} currents

The effects of azelastine on Ca^{2+} current can be characterized as follows: (i) direct reduction of both high-voltage-activated Ca^{2+} current (HVACC, $I_{Ca,\,L}$) and low-voltage-activated Ca^{2+} currents; (ii) no voltage dependence of current block; and (iii) no stereoselectivity of effect.

To elicit $I_{Ca,\,L}$, 200-ms-long pulses were applied from a holding potential of -100 to 0 mV under conditions where Ca^{2+} was the only charge carrier for inward current (Satoh and Horie, 1997; Heubach et al., 2000). This Ca^{2+} current was completely and reversibly blocked by 100 μM Cd^{2+} (data not shown, $n = 4$), indicating that it was not contaminated by K^+ and Na^+ currents (Ravens et al., 1989; McDonald et al., 1994). Azelastine was found to inhibit $I_{Ca,\,L}$ in a concentration-dependent manner with an IC_{50} of about 20 μM. At 100 μM, it almost completely blocked the L-type Ca^{2+} signals. Azelastine at 10 μM shifted the steady-state inactivation curve to the left by about 5 mV and enhanced the rate of slow inactivation. Moreover, the azelastine-induced block of $I_{Ca,\,L}$ did not show voltage dependence between -100 and -60 mV since the extent of block was similar at these two holding potentials. Thus, the mode of block did not resemble that of dihydropyridine derivatives (Porzig, 1990) confirming the results obtained with action potential shortening which, in contrast to effects of dihydropyridines, did exhibit reversed use-dependence. Moreover, we could not detect any stereoselectivity of drug action.

The current–voltage relation for Ca^{2+} currents clearly exhibited a low-voltage-activated component that reached its peak around -35 mV. Azelastine also reduced LVACC albeit with a similar potency to that observed for $I_{Ca,\,L}$, i.e. with an IC_{50} value of 24 μM for LVACC. The LVACC has been shown previously to be composed of T-type and tetrodotoxin-sensitive Ca^{2+} currents in guinea pig ventricular myocytes (Heubach et al., 2000). Although we did not distinguish between T-type and tetrodotoxin-sensitive Ca^{2+} currents in the present work, we assume that both components of LVACC were affected, since azelastine almost completely suppressed total current activated in the low-voltage range (Fig. 3A). Taken together with indirect data on block of Na^+ channels (i.e. reduced dV/dt_{max}; Molyvdas et al., 1989 and this paper), our results suggest that azelastine is a rather unselective ion channel blocker.

4.3. Clinical implications

To the best of our knowledge, no alterations of cardiac function have been reported so far in patients treated with azelastine for asthma bronchiale or allergic rhinitis (Davis, 1989). The effective therapeutic plasma concentrations were in the order of 10–100 nM/l (Tatsumi et al., 1980; McNeely and Wiseman, 1998), which is two to three orders of magnitude lower than the concentrations used in the present study. It may thus be concluded that adverse reactions due to Ca^{2+} channel blockade are very unlikely to occur in a clinical setting.

In conclusion, azelastine at micromolar concentrations is a Ca^{2+} channel blocker in guinea pig myocardial preparations; however, its mode of action is different from that of dihydropyridine derivatives. Lack of stereoselectivity of action and additional block of LVACC and Na^+ channels at concentrations 100–1000-fold higher than therapeutically relevant plasma concentrations suggest a rather unselective effect that may well include further ion channels. Our present results demonstrate that azelastine in concentrations used clinically appears safe with respect to cardiovascular Ca^{2+} channel antagonism.

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