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Tedisamil inhibits the delayed rectifier K^+ current in single smooth muscle cells of the guinea-pig portal vein

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Abstract. Tedisamil is a new bradycardic agent with an inhibitory action on K^+ channels in cardiac muscle, and secondary beneficial effects in experimentally induced cardiac ischemia. In whole-cell clamp studies in enzymatically dispersed, single smooth muscle cells from the guinea-pig portal vein, tedisamil inhibited the delayed rectifier K^+ current (determined as the charge transferred through the cell membrane), the mean concentration for half-maximal inhibition being 2.9 μM. In contrast to controls in the absence of drugs or in the presence of the classical K^+ channel blockers barium, tetraethylammonium or 4-aminopyridine, the time course of the delayed rectifier K^+ current in the presence of tedisamil could no longer be fitted by a single exponential, and signs of an accelerated inactivation by tedisamil were obtained. The slow onset of the response to tedisamil applied to the outside of the vascular myocytes, and the finding that tedisamil applied directly to the cytosol via the pipette was highly effective, suggest an intracellular site of action.

Key words: Cardiovascular agents – Patch-clamp technique – K^+ channel blockers – TEA – Barium – 4-aminopyridine

Introduction

Tedisamil {3,7-di-(cyclopropylmethyl)-9,9-tetramethylene-3,7-diazabicyclo[3.3.1]nonane dihydrochloride; KC 8857} is a new cardiovascular drug, the predominant action of which in vivo is the generation of bradycardia without negative inotropism [9]. Owing to this property, tedisamil was found to reduce the oxygen demand of the heart during exercise in dogs with an experimental coronary stenosis [5]. Its beneficial effect on ischaemia-induced arrhythmia has been studied in rats with experimental coronary occlusion, where it suppressed premature beats and reduced the occurrence of ventricular fibrillations [1]. From in vitro investigations in isolated cardiac myocytes it has been concluded that tedisamil acts by inhibition of repolarizing K^+ currents (transient outward and delayed rectifier currents), and hence induces a considerable prolongation of the action potential [1, 3, 4].

While in vivo, the cardiac actions of tedisamil obviously dominate, recent work in vitro on other tissues has revealed that its K^+ channel blocking activity is not strictly specific to cardiac tissue; in mouse astrocytes, two time-dependent and voltage-dependent K^+ currents (A-type and delayed-rectifier K^+ currents) were also found to be inhibited by tedisamil [4] and, in enzymatically dispersed cells of the guinea-pig portal vein, inhibition by tedisamil of single large-conductance Ca^{2+}-dependent K^+ channels (BK_{Ca}) was demonstrated by the patch-clamp technique [12]. Furthermore, in isolated aorta from rodents, tedisamil interferes with the ^{86}Rb efflux induced by the potassium channel opener cromakalim [2, 8] or by depolarization with high extracellular K^+ [8].

Following that previous work, the present electrophysiological study was performed to further characterize the K^+ channel blocking effects of tedisamil in vascular smooth muscle; for this purpose, the whole-cell clamp technique has been used in guinea-pig isolated portal vein cells. Some of the data have been presented previously in abstract form [7, 13].

Materials and methods

Vascular myocytes were prepared form guinea-pig portal veins with collagenase and papain as described previously [10, 11, 12]. The cells were superfused with bath solution (2 ml/min) at room temperature. Only cells responding to 1 μM noradrenaline were used. Membrane currents were measured in the whole-cell configuration [6] with an EPC-7 patch-clamp amplifier (List Electronics, Darmstadt), stored on hard disk or laser disk, and analysed by the pClamp software (version 5.5; Axon Instruments, Burlingame, USA). Values are given as means ± SEM; n = number of observations.

Tedisamil (KC 8857) was kindly supplied by Kali-Chemie (Hannover, FRG). Bath solution contained (in mM) NaCl 128.0, $NaHCO_3$ 14.4, KCl 4.7, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $Na_2CaEDTA$ 0.1, $CaCl_2$ 1.5, glu-

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cose 10, HEPES 10 (adjusted to pH 7.2 by approximately 5 mM NaOH). The pipette solution contained: KCl 70, KH_2PO_4 30, $MgSO_4$ 5, Na_2ATP 5, sodium creatine phosphate 5, sodium pyruvate 5, sodium β-hydroxybutyrate 5, taurine 20, EGTA 2, $CaCl_2$ 0.2 (free $[Ca^{2+}] = 20$ nM), KOH approximately 40, pH 7.2.

Results

Characterization of the whole-cell outward currents

On voltage jumps from −60 mV to values more positive than −20 mV, the guinea-pig portal vein cells exhibited an outward current, which partly inactivated with time (Figs. 2 and 3, original tracings). At +40 mV, the outward current reached a peak of 309±44 pA ($n = 11$) within approximately 200 ms. Withdrawal of extracellular calcium (2 mM EGTA added to the bath solution) did not obviously alter the outward current.

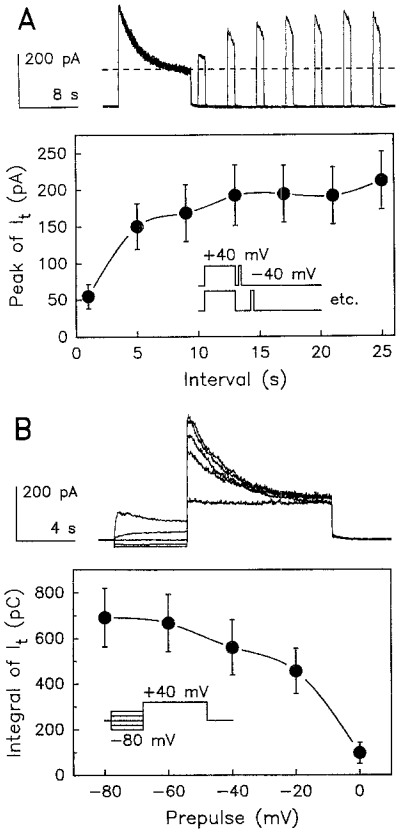

Fig. 1 A, B. Kinetics of the transient current I_t measured in guinea-pig isolated portal vein cells by the whole-cell clamp technique. **A** Time course of the recovery from inactivation of I_t. *Upper graph*: superposition of seven current traces elicited by prepulses from the holding potential (−40 mV) to +40 mV for 10 s, each followed by a test pulse to +40 mV applied for 1 s at variable interpulse intervals rising from 1 s to 25 s. Time between the start of traces = 60 s. The broken line indicates the limit of discrimination for the determination of I_t and I_{ss}. *Lower graph*: relationship between the peak I_t during the test pulse and the interpulse interval derived from the same type of experiments. Means±SEM, $n = 5$. **B** Dependence of I_t on the voltage of the prepulse (steady-state inactivation). *Upper graph*: experiment using a single cell. The lower traces during the prepulses belong to the upper traces during the test pulse, and vice versa. The time between the start of the traces was 35 s. *Lower graph*: same type of experiment, means±SEM, $n = 5$. I_t determined as the charge transferred during the test pulse

For subsequent evaluations, the total outward current was subdivided into its two components, i.e. into the transient current (I_t) and the time-independent steady-state current (I_{ss}). For this purpose, the steady-state current I_{ss} was determined at the end of the test pulses, i.e. when I_t had settled; in Fig. 1 A (upper graph), the broken line indicates how, for subsequent data evaluation, the two components of the outward current were discriminated. Since the mean seal resistance in the cell-attached configuration was 14.3±1.2 GΩ ($n = 10$), and since at +40 mV, the estimated leak current via the seal resistance was less than 10 pA, it seemed unnecessary to correct I_{ss} for leak currents.

The time course of the inactivation of I_t could be fitted with a mono-exponential function (at +40 mV, $\tau = 2.45 \pm 0.12$ s, $n = 11$) indicating the apparently unitary nature of I_t. After a voltage jump to +40 mV for 10 s and the subsequent return to the holding potential of −40 mV, I_t took several seconds to revover from its inactivation (Fig. 1 A).

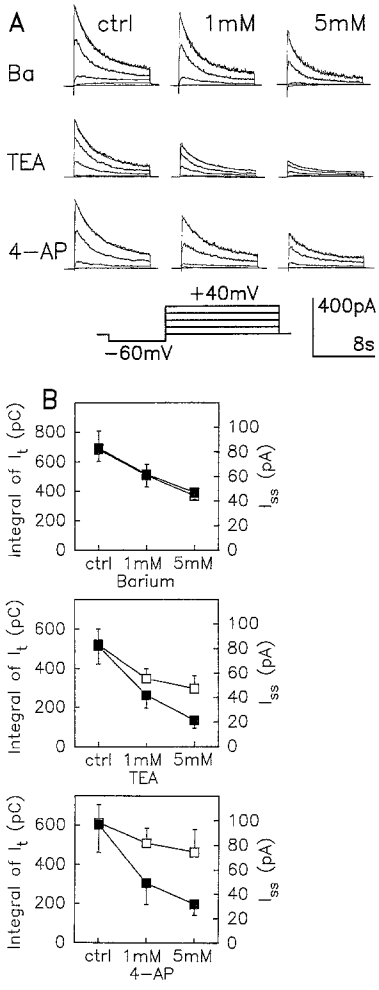

Fig. 2 A, B. Inhibition of I_t and I_{ss} by 1 mM and 5 mM Ba^{2+}, tetraethylammonium (*TEA*) and 4-aminopyridine (*4-AP*) respectively. **A** Currents elicited by test pulses from −40 to +40 mV after a prepulse of −60 mV (current tracings during prepulses omitted). The traces at +40 mV are superimposed by mono-exponential fitting curves. **B** Inhibition of I_t (■) and I_{ss} (□) at a test pulse of +40 mV. *y* axes chosen for congruence of the contol (*ctrl*) values; means±SEM, $n = 5$

As a quantity that takes into account both the amplitude and the time course of I_t, we used the charge Q_t, i.e. the time integral of I_t (in pC = pA×s), transferred during a voltage jump to +40 mV for 10 s. Figure 1B shows the dependence of Q_t on the voltage of a prepulse of 5 s duration. Q_t was larger after more negative prepulses, and was nearly abolished with a prepulse of 0 mV.

To assess the ion species underlying I_t, experiments were performed with the classical K^+ channel blockers Ba^{2+}, tetraethylammonium (TEA) and 4-aminopyridine in concentrations of 1 mM and 5 mM each (Fig. 2). At 5 mM, these blockers reduced Q_t to 55%, 26% and 33% of the control value respectively. They did not significantly modify the mono-exponential time course of the inactivation of I_t (Fig. 2A; at +40 mV and a 5 mM concentration of the blockers, the time constants τ were calculated to be 2.94±0.28 s ($n = 5$) for barium, 2.69±0.05 s ($n = 5$) for TEA, and 2.77±0.06 s ($n = 5$) for 4-aminopyridine ($n = 5$). Compared with the above control value (2.45±0.12 s) by Student's t-test, t values between 1.249 and 1.983, at 14 degrees of freedom, were obtained, which were all non-significant (2 $P > 0.05$ and < 0.3).

The steady-state current I_{ss} was differently affected by the K^+ channel blockers applied. The scales of the y axes in Fig. 2B were chosen for congruence of the control values of I_t (expressed as Q_t) and I_{ss} at +40 mV. Ba^{2+} reduced the two components approximately to the same degree, whereas TEA, and 4-aminopyridine to an even greater extent, had a stronger effect on Q_t.

Effect of tedisamil on transient and steady-state outward currents

Tedisamil, from 1 µM to 10 µM, reduced both currents, but I_t to a greater extent than I_{ss} (Fig. 3). At +40 mV, 100 µM tedisamil reduced Q_t to 1.4% and I_{ss} to 63% of their respective control values (Fig. 3B, C). For Q_t, the IC_{50} of tedisamil (the concentration that reduced Q_t to 50% of controls) was 2.9−0.5/+0.6 µM ($n = 5$), while for I_{ss}, the IC_{50} was greater than 100 µM.

At submaximal concentrations of tedisamil, the inactivation of I_t no longer followed a mono-exponential time course; in contrast to the other K^+ channel blockers (Fig. 2A), a fast and a slow component became visible (Fig. 3A). At 10 µM tedisamil and a voltage jump to +40 mV, the calculated time constant of the fast component was 0.086±0.021 s, and that of the slow component, 9.06±0.52 s ($n = 5$).

Table 1. Inhibition of Q_t in guinea-pig isolated portal vein cells measured by whole-cell clamping from −60 mV to +40 mV for 10 s by tedisamil either applied to the bath side (extracellular application) or from within the pipette (intracellular application)[a]

Tedisamil (µM)	Extracellular application			Intracellular application		
	Q_t (pC)	n	$2P$	Q_t (pC)	n	$2P$
1	359±113	5	>0.3	227±33	5	>0.1
10	110± 30	5	<0.02	73±35	5	<0.01
0 (control)	490± 83	14				

[a] Results are means±SEM. Probability calculated by Student's t-test for unpaired data, two-sided

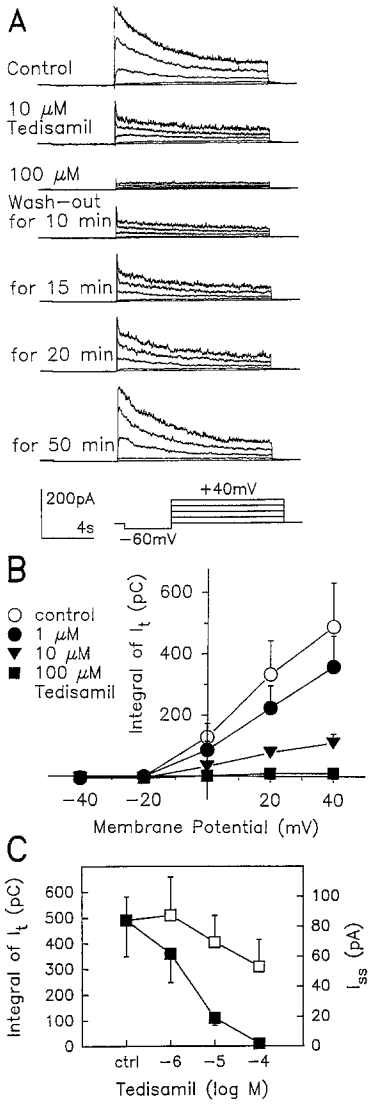

Fig. 3A–C. Effects of different concentrations of tedisamil on I_t and I_{ss} in guinea-pig isolated portal vein cells. **A** Original traces. Note reductions and alteration of shape of current tracings, and slow recovery on washout of the drug. **B** Relationship of I_t (determined as Q_t) and membrane potentials evoked by test pulses and inhibitory effect of 1−100 µM tedisamil (means±SEM, $n = 5$). **C** Inhibition by tedisamil of I_t (■) and I_{ss} (□) at a test pulse of +40 mV. y axes chosen for congruence of the control values; means±SEM, $n = 5$

Both the onset of and the recovery from the effect of tedisamil on I_t were slow (Fig. 4). With TEA, blockade of I_t was almost complete within 1 min, and its recovery became visible after 1 min. In contrast, it took 5 min for the full inhibitory effect of tedisamil to register, while recovery of I_t did not become visible until 3 min after the start of the washout.

When tedisamil (1 µM or 10 µM) was added to the pipette solution and thus admitted directly to the cytosolic side of the cell membrane, while the extracellular surface was rinsed continuously with drug-free bath solution at 2 ml/min, it was as effective an inhibitor of Q_t as when admitted extracellularly (Table 1). The effect was regularly found complete within the short period (i.e. 50−100 s) from establishing the whole-cell configuration by suction until the start of the electrical recording.

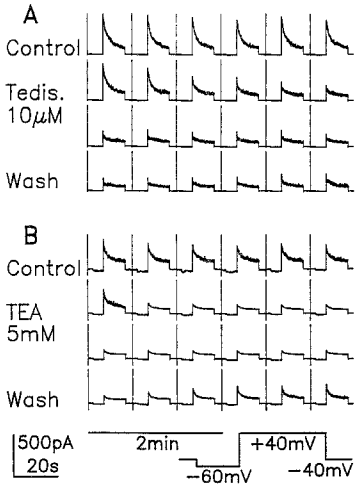

Fig. 4A, B. Comparison of the time dependence of the onset of and the recovery from the inhibitory action of tedisamil (**A**) and of TEA (**B**) on I_t in guinea-pig isolated portal vein cells. Original tracings; drugs admitted in traces 2 and 3 of either graph. Single episodes lasted 20 s and were followed by a 20-s intermission (not shown)

Discussion

In previous work from this laboratory we have shown that tedisamil reduces the open-probability of large-conductance Ca^{2+}-dependent K^+ channels (BK_{Ca}) in guinea-pig portal vein cells [12]. Here we have demonstrated that in single cells of the same vascular tissue, tedisamil interfered with an outwardly directed, time- and voltage-dependent K^+ current in the same type of cells. This effect was obtained at concentrations two to three orders of magnitude smaller than those of the classical K^+ channel blockers.

The transient current observed in our study had the typical properties of a delayed rectifier K^+ current, i.e. (a) activation on voltage jumps to 0 mV or higher, (b) a slow rise and inactivation, (c) steady-state inactivation complete at 0 mV, (d) recovery from inactivation established after approximately 15 s, and (e) Ca^{2+} independence [14]. A current with these properties (but labelled as I_{to}) has been described in detail recently by Noack et al. [11], also in guinea-pig portal vein cells.

This current was inhibited by tedisamil at an IC_{50} of 2.9 μM, which is in the range of the dissociation constants (2–5 μM) given by Dukes et al. [4] for the binding of tedisamil to various time- and voltage-dependent K^+ channels in heart and glial cells. To block the BK_{Ca} channel of the guinea-pig portal vein, a higher concentration was required (IC_{50} = 14 μM; [12]). The inhibitory effect of tedisamil on the steady-state current was less prominent than on the delayed rectifier current. The precise nature of the steady-state current has to be established; the differential effects of tedisamil and of the other blockers tested in this study suggest that different channel populations may underlie I_t and I_{ss}. Of course, the possibility cannot be excluded that I_t and I_{ss} are mediated by the same channels reacting differently to the same blockers because of time-dependent behaviour.

In contrast to our observations in inside-out patches [12] and to the data obtained with TEA in the present study, the actions of tedisamil in the whole-cell configuration had a slow onset (full inhibition in inside-out patches: 1 min; in the whole-cell configuration: 5 min) and decline (inside-out: complete recovery within 2 min; whole-cell: 30–45 min), suggesting an intracellular site of action. Also, tedisamil applied to the cytosolic side of the vascular smooth muscle cells was an effective K^+ channel blocker in our study, thus confirming the previous suggestion of Dukes et al. ([4] in cardiac myocytes) that tedisamil acts from the intracellular side on the K^+ channels.

In the same study [4], it was stated that tedisamil blocked voltage-dependent K^+ currents "by a process resembling accelerated inactivation". In our study, while the inactivation of the delayed rectifier occurred at a considerably lower rate than in Dukes' study, tedisamil modified its inactivation time course in a similar way.

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