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Quercetin as a novel activator of L-type Ca^{2+} channels in rat tail artery smooth muscle cells

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1 The aim of this study was to investigate the effects of quercetin, a natural polyphenolic flavonoid, on voltage-dependent Ca^{2+} channels of smooth muscle cells freshly isolated from the rat tail artery, using either the conventional or the amphotericin B-perforated whole-cell patch-clamp method.

2 Quercetin increased L-type Ca^{2+} current [$I_{Ca(L)}$] in a concentration- ($pEC_{50} = 5.09 \pm 0.05$) and voltage-dependent manner and shifted the maximum of the current-voltage relationship by 10 mV in the hyperpolarizing direction, without, however, modifying the threshold and the equilibrium potential for Ca^{2+}. Quercetin-induced $I_{Ca(L)}$ stimulation was reversible upon wash-out. T-type Ca^{2+} current was not affected by quercetin.

3 Quercetin shifted the voltage dependence of the steady-state inactivation and activation curves to more negative potentials by about 5.5 and 7.5 mV respectively, in the mid-potential of the curves as well as increasing the slope of activation. Quercetin slowed both the activation and the deactivation kinetics of the $I_{Ca(L)}$. The inactivation time course was also slowed but only at voltages higher than 10 mV. Moreover quercetin slowed the rate of recovery from inactivation.

4 These results prove quercetin to be a naturally-occurring L-type Ca^{2+} channel activator.
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Keywords: L-type Ca^{2+} channel activator; quercetin; rat tail artery smooth muscle; whole-cell patch-clamp

Abbreviations: Bay K 8644, (S)-methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxilate; BHA, 3-*t*-butyl-4-hydroxyanisole; CGP 28392, ethyl-4(2-difluoromethoxyphenyl)-1,4,5,7-tetrahydro-2-methyl-5-oxofuro-[3,4-b] pyridine-3-carboxylate; DTBHA, 3,5-di-*t*-butyl-4-hydroxyanisole; E_{Ca}, equilibrium potential for Ca^{2+}; FPL 64176, methyl 2,5-dimethyl-4-[2-(phenylmethyl)benzoyl]-1H-pyrrole-3-carboxylate; G, conductance; $I_{Ca(L)}$, L-type Ca^{2+} currrent; $I_{Ca(T)}$, T-type Ca^{2+} current; PSS, physiological salt solution; RS 30026, methyl 2-methyl-4-(2-benzyloxyphenyl)-5-oxo-1,4,5,7-tetrahydrothieno [3,4-b] pyridine-3-carboxylate; TEA, tetraethylammonium; V_h, holding potential

Introduction

Quercetin is a natural polyphenolic flavonoid widely found in edible plants (i.e. fruits, vegetables, herbs, grains) and beverages (i.e. tea, red wine). Interest in dietary phenolics has increased greatly recently, owing to their antioxidant properties (free radical scavenging and metal chelating) and their possible beneficial implications in human health, such as in the treatment and prevention of cancer, cardiovascular diseases, and other pathologies (Bravo, 1998). Epidemiological studies have revealed that the Mediterranean diet, being based primarily on flavonoid-rich foods, correlates with increased longevity (Orgogozo *et al*., 1997) and decreased incidence of cardiovascular diseases (Hertog *et al*., 1993; 1995; Renaud *et al*., 1998; 1999).

Quercetin has been shown to possess biological properties responsible for its beneficial effects on the cardiovascular system. For example, it modifies eicosanoid biosynthesis (antiprostanoid and anti-inflammatory responses), protects low-density lipoprotein from oxidation (preventing athero-sclerotic plaque formation), prevents platelet aggregation (antithrombic effects), and promotes relaxation of vascular smooth muscle (antihypertensive effect) (Formica & Regelson, 1995). In addition, oral administration of quercetin to spontaneously hypertensive rats has recently been shown to produce antihypertensive effects (Duarte *et al*., 2001).

Until now, the effects of this flavonoid on the electrophysiological properties of vascular smooth muscle Ca^{2+} channels has not been investigated, although a decreased transmembrane Ca^{2+} influx (Formica & Regelson, 1995) was indicated as a possible mechanism for its relaxing and vasodilator effects. However, Summanen *et al*. (2001) have recently demonstrated that quercetin increases Ca^{2+} current (I_{Ca}) in clonal rat pituitary GH_4C_1 cells.

Therefore, the aim of this study was to investigate the effects of quercetin on voltage-dependent Ca^{2+} channels in single smooth muscle cells isolated from the rat tail main artery in an attempt to clarify whether quercetin might modulate vascular I_{Ca} and whether this effect might be related to its myorelaxing properties.

The results here presented suggest that quercetin is an L-type Ca^{2+} channel activator. A preliminary account of the effects of quercetin on rat tail $I_{Ca(L)}$ was presented at the 30th National Meeting of the Italian Pharmacological Society (Saponara *et al*., 2001).

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Methods

Cell isolation procedure

Smooth muscle cells were freshly isolated from the tail main artery of male rats (350–450 g) by means of collagenase treatment, as previously described (Fusi *et al*., 2001b). The cells were continuously superfused with physiological salt solution (PSS) using a peristaltic pump (LKB 2132) at a flow rate of 800 $\mu l \times min^{-1}$. Electrophysiological responses were tested at room temperature (22–24°C) only in those cells that were phase dense.

Whole-cell patch clamp recording

Conventional (Hamill *et al*., 1981) and amphotericin B-perforated (Rae *et al*., 1991) whole-cell patch-clamp methods were employed to voltage-clamp smooth muscle cells. Recording electrodes were pulled from borosilicate glass capillaries (WPI, Berlin, Germany) and fire-polished to create a pipette resistance

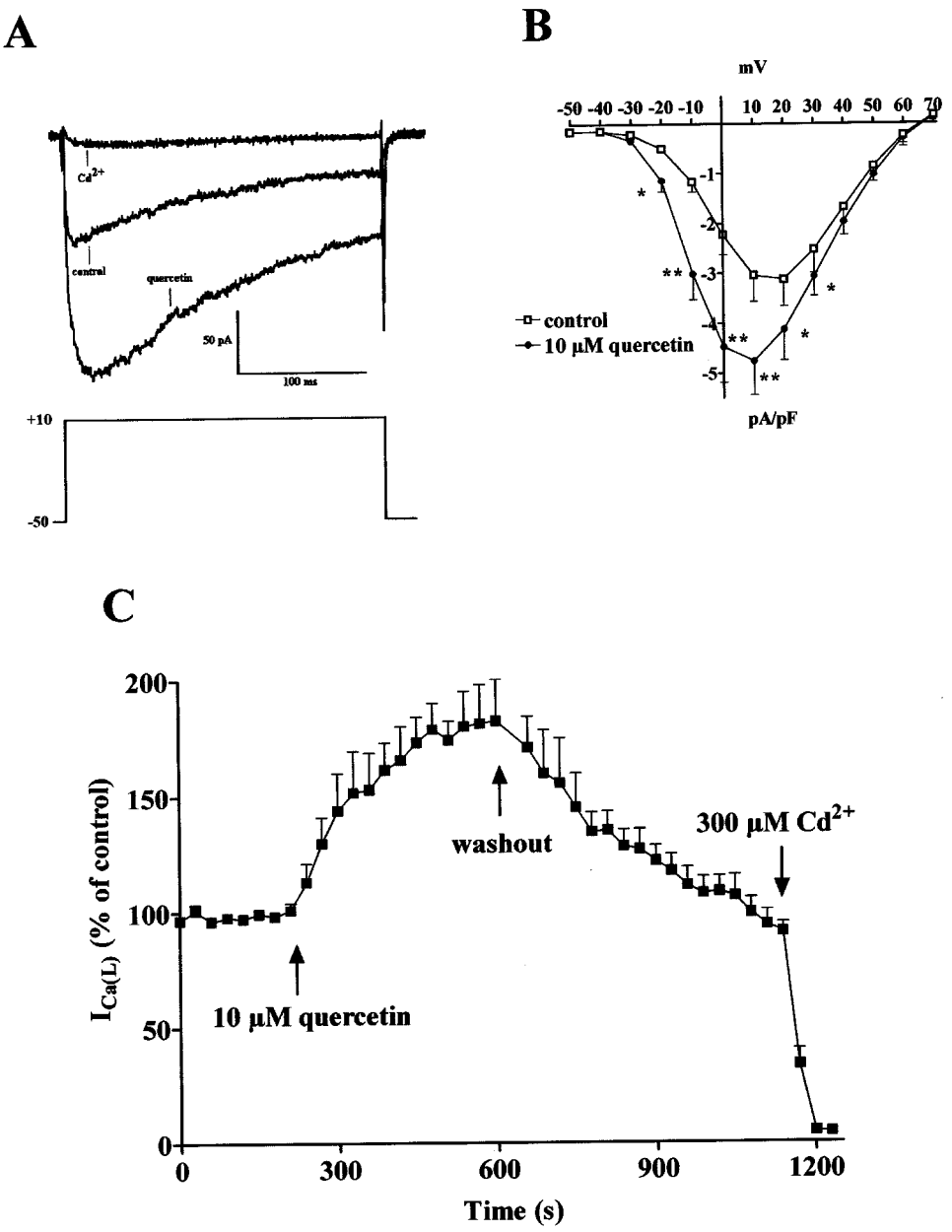

Figure 1 Stimulation by quercetin of $I_{Ca(L)}$. (A) Original recordings of conventional whole-cell $I_{Ca(L)}$ in rat tail artery myocytes elicited with 250-ms clamp pulses to 10 mV from a V_h of −50 mV (see schematic diagram), measured in the absence (control) or presence of quercetin (10 μM). $I_{Ca(L)}$ suppression by 300 μM Cd^{2+} is also shown. (B) Current-voltage relationships constructed prior to the addition of quercetin (control) and in the presence of 10 μM quercetin. Data points are mean±s.e.mean of five cells ($n=4$). $^*P<0.05$, $^{**}P<0.01$, Student's *t*-test for paired samples. (C) Time course of $I_{Ca(L)}$ activation induced by quercetin. Quercetin (10 μM) was applied, at the time indicated by the arrow, and peak currents were recorded during a typical depolarization from −50 to 10 mV, applied every 30 s, and subsequently normalized according to the current recorded just prior to quercetin application. Drug wash-out allows for full recovery of stimulation. Data points are mean±s.e.mean of four cells ($n=3$).

of 2–5 MΩ when filled with internal solution. A low-noise, high-performance Axopatch 200B (Axon Instruments, U.S.A.) patch-clamp amplifier driven by an IBM computer in conjunction with an A/D, D/A board (DigiData 1200 A/B series interface, Axon Instruments, U.S.A.) was used to generate and apply voltage pulses to the clamped cells and record the corresponding membrane currents. Current signals, after compensation for whole-cell capacitance, series resistance and liquid junction potential, were low-pass filtered at 1 kHz and digitized at 3 or 10 kHz prior to being stored on the computer hard disk. I_{Ca} was always recorded in 5 mM Ca^{2+}-containing PSS: this concentration was shown to cause maximal peak current in arterial smooth muscle cells (Bolton *et al*., 1988).

I_{Ca} was measured over a range of test potentials (250 ms) from −50 to 70 mV from a holding potential (V_h) of −50 or −90 mV. Data were collected once the current amplitude had been stabilized (usually 5–10 min after the whole-cell configuration had been obtained). I_{Ca} did not run down during the following 30–40 min under these conditions.

A two-pulse protocol was applied to record T-type Ca^{2+} currents ($I_{Ca(T)}$). The cell was first depolarized for 250 ms to −40 mV from a V_h of −90 mV to elicit $I_{Ca(T)}$. Following a 6 s return to the V_h, a 250-msec clamp pulse to 10 mV was applied to record $I_{Ca(L)}$, taken as 100%.

Steady-state inactivation curves were obtained using the double-pulse protocol. Once various levels of the conditioning potential had been applied for 5 s, followed by a short (5 ms) return to the holding potential, a test pulse (250 ms) to 10 mV was delivered to evoke the current.

Activation curves were derived from the current-voltage relationships (see Figure 1B). Conductance (G) was calculated from the equation $G = I_{Ca}/(E_m - E_{Ca})$, where I_{Ca} is the peak current elicited by depolarizing test pulses from −50 to 30 mV from V_h of −50 mV and E_{Ca} is the equilibrium potential for Ca^{2+}. G_{max} is the maximal Ca^{2+} conductance (calculated at potentials above 10 mV). The points for G/G_{max} were plotted against the membrane potential as relative amplitude.

A two-pulse protocol was applied to measure the time course of recovery from inactivation: 2-s clamp pulses to 10 mV from a V_h of −50 mV were followed by a return to the V_h of variable duration to allow some channels to recover from inactivation. A second pulse (250 ms) to 10 mV was delivered to determine how much recovery had occurred during the time interval.

K^+ currents were blocked with 30 mM tetraethylammonium (TEA) in the PSS and Cs^+ in the internal solution.

Current values were corrected for leakage using 300 μM Cd^{2+} which was assumed to completely block I_{Ca}. Tail currents were leak subtracted using the P/4 protocol.

Solutions and chemicals

Ca^{2+}-free PSS contained (in mM): NaCl 110; KCl 5.6; HEPES 10; taurine 20; glucose 20; $MgCl_2$ 1.2; Na-pyruvate 5 (pH 7.4).

The internal solution for the conventional method (pCa 8.4) consisted of (in mM): CsCl 100, HEPES 10, EGTA 11, $MgCl_2$ 2, $CaCl_2$ 1, Na-pyruvate 5, succinic acid 5, oxalacetic acid 5, Na_2-ATP 3 and 5-phosphocreatine; pH was adjusted to 7.4 with CsOH. For the perforated method, the internal solution (pCa 8.4) contained (in mM): CsCl 125, HEPES 10, EGTA 11, $MgCl_2$ 2, $CaCl_2$ 1, amphotericine B (200 μg ml^{-1}); pH was adjusted to 7.4 with CsOH.

Amphotericin B (100 mg ml^{-1}) was first dissolved in dimethylsulphoxide (DMSO) and then added to the internal solution.

The osmolarity of PSS was adjusted to 335 mosmol and that of the internal solution to 310 mosmol (Stansfeld & Mathie, 1993) by means of an osmometer (Osmostat OM 6020, Menarini Diagnostics, Italy).

The chemicals used were: collagenase (type XI), TEA, bovine serum albumin, trypsin inhibitor, amphotericin B, $CdCl_2$, (S)-methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)pyridine-5-carboxilate (Bay K 8644) and quercetin (Sigma Chimica, Italy). Quercetin dissolved directly in DMSO, and Bay K 8644, dissolved in ethanol, were diluted at least 1000 times in PSS, prior to use. The resulting concentrations of DMSO and ethanol (below 0.1%) failed to alter the current (data not shown). Final drug concentrations are presented in the text.

Following control measurements, each cell was drug exposed by perfusing the experimental chamber with a drug-containing PSS.

Statistical analysis

Acquisition and analysis of data were accomplished using pClamp 8.0.1.12 software (Axon Instruments, U.S.A.) and GraphPad Prism version 3.02 (GraphPad Software, U.S.A.). Data are reported as mean ± s.e.mean; n is the number of animals (indicated in parentheses). Statistical analyses and significance as measured by the Student's t test for unpaired and paired samples were obtained using GraphPad InStat version 3.02 (GraphPad Software, U.S.A.) as appropriate. In all comparisons, $P<0.05$ was considered significant. The current-voltage relationships were calculated on the basis of the peak values (leakage corrected) from the original currents.

Results

Effects of quercetin on $I_{Ca(L)}$: conventional whole-cell method

Figure 1A shows a typical recording of $I_{Ca(L)}$ under control conditions and following the addition of 10 μM quercetin as well as quercetin plus 300 μM Cd^{2+}. The current voltage relationship (Figure 1B) showed that quercetin significantly increased the peak inward current in the range −20 to 30 mV, and shifted the maximum by 10 mV in the hyperpolarizing direction without, however, varying the threshold at about −30 mV. E_{Ca} obtained under control conditions (63.71 ± 2.37 mV) was not modified by 10 μM quercetin (64.92 ± 1.18 mV; four cells, $n=3$). Furthermore, quercetin stimulated $I_{Ca(L)}$ in a concentration-dependent manner with a pEC_{50} of 5.09 ± 0.05 μM (five cells, $n=4$). The increase in maximum $I_{Ca(L)}$ recorded at 10 mV observed in presence of 50 μM quercetin (226.8 ± 37.3%; five cells, $n=3$) was comparable to that recorded in the presence of 100 nM Bay K 8644 under the same experimental conditions (291.7 ± 29.0%; nine cells, $n=5$).

Figure 1C shows the time course of quercetin's effect on the current. Once $I_{Ca(L)}$ had reached steady values, quercetin (10 μM) was added to the bath solution. This produced a gradual increase of the current that reached a plateau in about 5 min and was reversible upon washout.

Effects of quercetin on $I_{Ca(L)}$: amphotericin B-perforated method

Maximum inward current recorded with the perforated method (-0.49 ± 0.04 pA/pF; six cells, $n=3$) was significantly lower than that recorded with the conventional method (-3.04 ± 0.53 pA/pF; five cells, $n=4$; $P<0.001$, Student's *t*-test for unpaired samples). This may arise from a mere technical reason, since the series resistance measured in the perforated method (12.2 ± 0.15 MΩ; six cells, $n=3$) was significantly higher than that in the conventional method (6.3 ± 0.13 MΩ; four cells, $n=3$; $P<0.05$, Student's *t*-test for unpaired samples). Nevertheless, with the perforated method, 10 μM quercetin significantly increased $I_{Ca(L)}$ in a manner similar to that observed with the conventional method. In fact, the current–voltage relationship was shifted to more negative potentials (data not shown) and the increase in maximum $I_{Ca(L)}$ recorded at 10 mV ($185 \pm 14.6\%$; four cells, $n=3$) was not significantly different from that recorded with the conventional configuration ($159.2 \pm 11.3\%$; five cells, $n=4$).

Effects of quercetin on $I_{Ca(T)}$

Cells expressing both T- and L-type Ca^{2+} channels were employed in this series of experiments (see Petkov *et al.*,

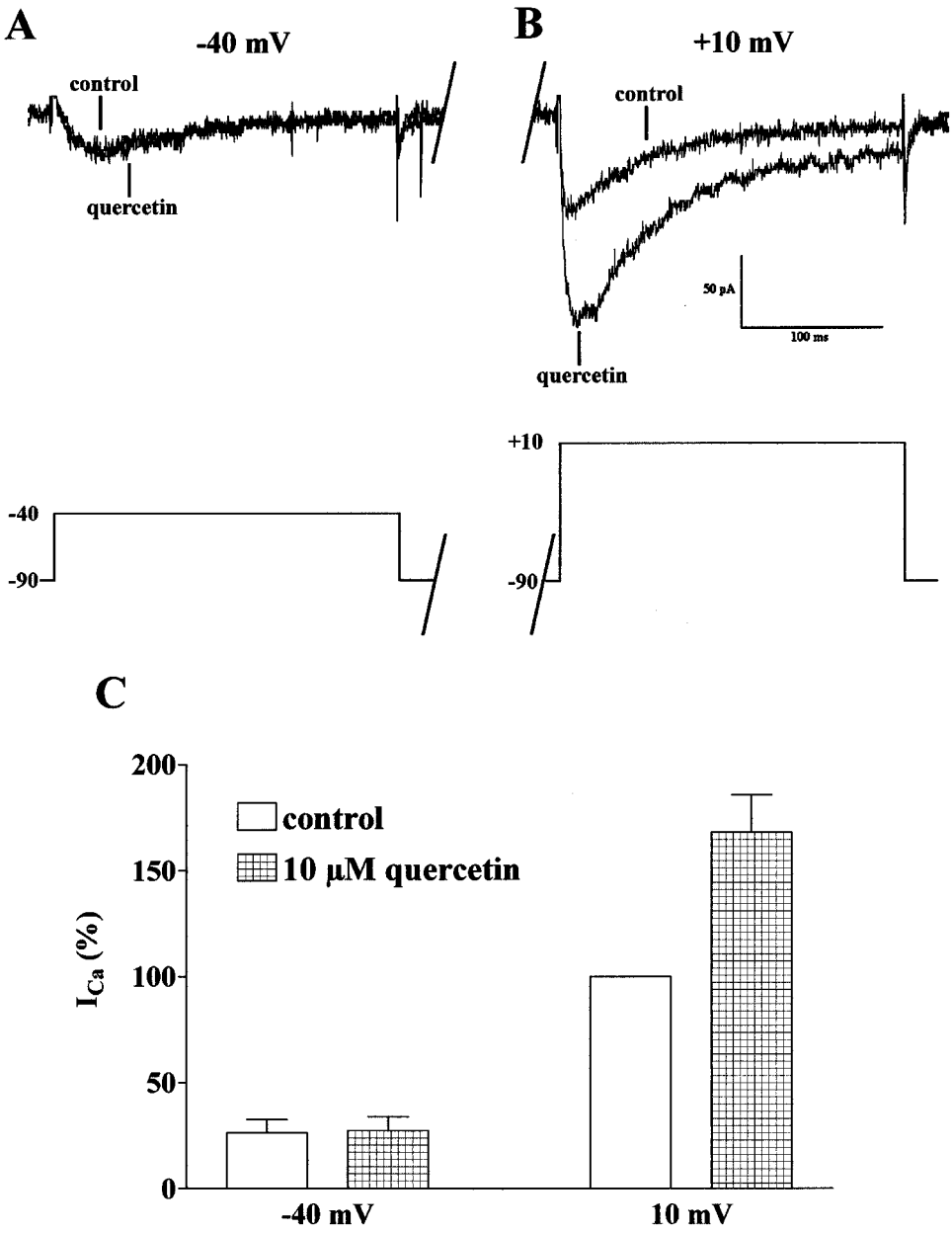

Figure 2 The effects of quercetin on $I_{Ca(T)}$. As shown below current traces, a two-pulse protocol was applied to record $I_{Ca(T)}$ and $I_{Ca(L)}$. Original recordings of conventional whole-cell I_{Ca} in rat tail artery myocytes elicited with 250-ms clamp pulses to −40 (A) and 10 mV (B) from a V_h of −90 mV (6-s interpulse every 30 s) measured in the absence (control) or presence of quercetin (10 μM). The two traces in panel (A) are superimposed. (C) The currents measured during the depolarizing pulse to both −40 and 10 mV from a V_h of −90 mV were expressed as a percentage of the current evoked by clamp pulse to 10 mV under control conditions. Each bar represents the mean ± s.e.mean of five cells ($n=4$).

2001). I_{Ca} recorded at −40 mV from a V_h of −90 mV was taken as an indicator of $I_{Ca(T)}$. This current, in fact, was no longer observed when V_h was shifted to −50 mV (data not shown). As shown in Figure 2B, 10 μM quercetin increased $I_{Ca(L)}$ (recorded at 10 mV) with a potency similar to that observed in the cells kept at V_h of −50 mV (see Figure 1B). On the contrary $I_{Ca(T)}$ was not significantly modified by quercetin (Figure 1A,C) up to a final concentration of 100 μM (data not shown).

Effects of quercetin on deactivation of $I_{Ca(L)}$

Under control conditions, following a 25-ms test pulse to 10 mV from a V_h of −50 mV, tail currents at −20 mV were analysed (Figure 3). Fitting of the trace revealed two time constants (τ): a fast component (τ_{fast}) of 1.29±0.15 ms and a much slower component (τ_{slow}) of 24.3±3.80 ms (eight cells, $n=4$). In the presence of 10 μM quercetin, τ_{fast} was significantly prolonged to 3.87±0.40 ms ($P<0.001$, Student's *t*-test for paired samples) whereas τ_{slow} remained unaltered (27.0±5.49 ms).

Effects of quercetin on steady-state inactivation and activation curves for $I_{Ca(L)}$

The voltage dependence of quercetin stimulation was analysed by determining the steady-state inactivation and activation curves for $I_{Ca(L)}$. Quercetin 10 μM significantly shifted the steady-state inactivation curve to more negative potentials (Figure 4A). The 50% inactivation potentials, evaluated by means of Boltzmann fitting, were −21.92±1.61 mV (control) and −27.52±1.76 mV (quercetin; five cells, $n=4$; $P<0.01$, Student's *t*-test for paired samples), respectively. On the contrary, the slope factor (−7.67±0.49 mV, control) was not affected by quercetin (−6.59±0.31 mV). Noticeably, the $I_{Ca(L)}$ stimulation produced by quercetin was larger at negative potentials and became progressively smaller as the conditioning potentials were increased (Figure 4B). In fact, after conditioning pulse to −80 mV for 5 s, $I_{Ca(L)}$ continued to be augmented to 155.02±13.7% by the presence of 10 μM quercetin.

The activation curves obtained from the current–voltage relationships in Figure 1 and fitted to the Boltzmann equation, are shown in Figure 4A. Quercetin (10 μM) significantly decreased the 50% activation potentials from −0.97±2.69 mV (control) to −8.41±1.15 mV (quercetin;

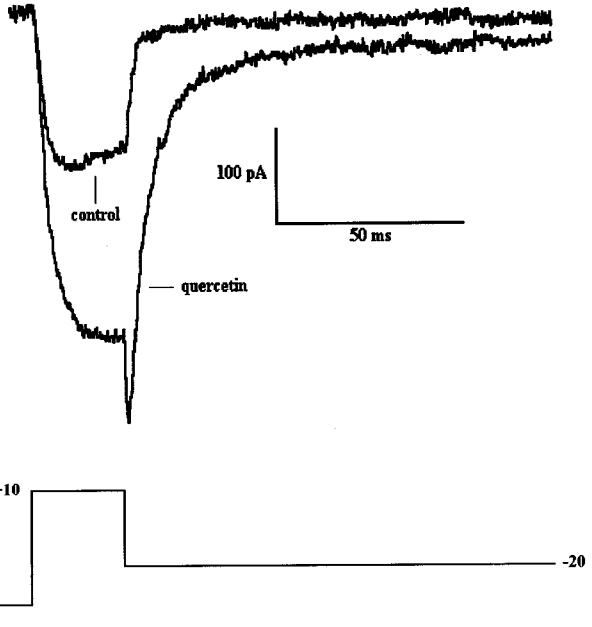

Figure 3 The effects of quercetin on Ca^{2+} channel tail current. Original recordings of conventional whole-cell $I_{Ca(L)}$ in rat tail artery myocytes elicited by 25-msec clamp pulse to 10 mV from a V_h of −50 mV, measured in the absence (control) or presence of quercetin (10 μM). Tail currents on repolarization to −20 mV were sampled at 10 kHz.

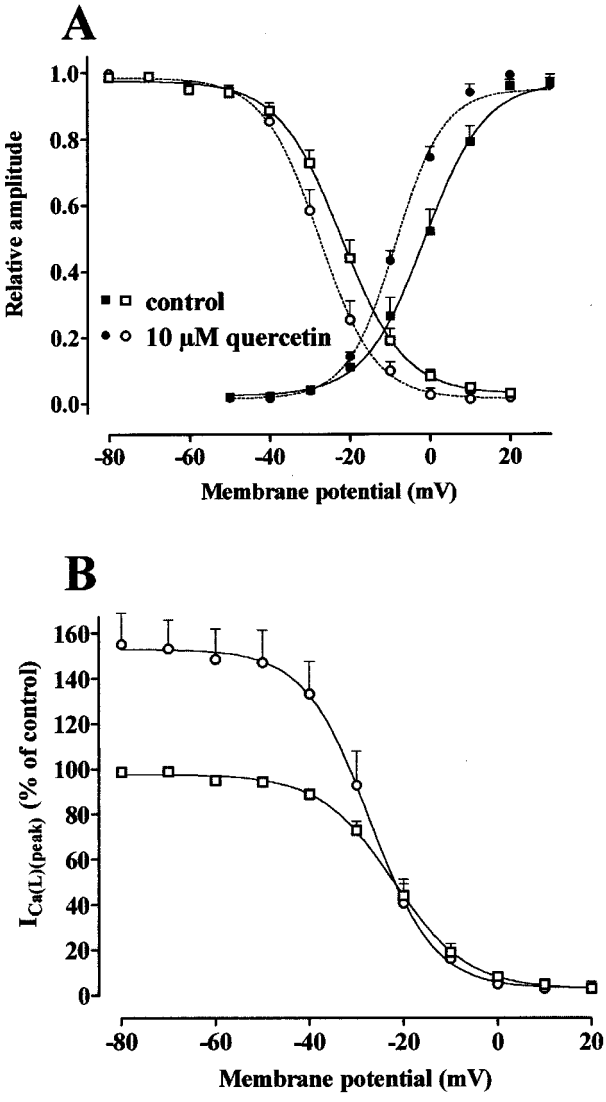

Figure 4 The effects of quercetin on both the activation and inactivation curves. Steady-state inactivation curves, obtained in the absence (control) and presence of 10 μM quercetin, were fitted to the Boltzmann equation. Peak current values were used. The steady-state inactivation curve was obtained using the double–pulse protocol (see Methods section). The current measured during the test pulse is plotted against membrane potential and expressed as relative amplitude (A) and in per cent of control (B). Activation curves were obtained from the current–voltage relationships of Figure 1B and fitted to the Boltzmann equation (see Methods section). Each point represents the mean±s.e.mean of five cells ($n=4$).

five cells, $n=4$; $P<0.05$, Student's t-test for paired samples), as well as the slope factors from 7.13 ± 0.48 mV (control) to 6.07 ± 0.43 mV (quercetin; $P<0.05$, Student's t-test for paired samples).

Effects of quercetin on $I_{Ca(L)}$ activation and inactivation kinetics

The kinetic of current activation and inactivation was studied in traces recorded from a V_h of -50 mV to test potentials of -20 to 50 mV. In control condition, the τ for activation, evaluated by single exponential fitting, decreased from -20 to 0 mV then remaining relatively unchanged up to 50 mV (Figure 5C). In the presence of 10 μM quercetin, the τ for activation, was significantly prolonged at all membrane voltages, and showed a bell-shaped pattern.

The fitting of the $I_{Ca(L)}$ inactivation rate revealed a mono-exponential τ value which was significantly prolonged by quercetin only at voltages more positive than 10 mV (Figure 5D).

Effects of quercetin on recovery from inactivation

The effect of quercetin on recovery from inactivation due to conditioning pulses to 10 mV is presented in Figure 6. Quercetin (10 μM) significantly slowed the rate of recovery from inactivation. Furthermore, mono-exponential fitting of the plot showed τ values of 406.60 ± 25.74 ms (control) and 627.98 ± 5.08 ms (quercetin; six cells, $n=5$; $P<0.05$, Student's t-test for paired samples), respectively.

Discussion

This study provides the first direct electrophysiological evidence that quercetin, a naturally-occurring flavonoid widely represented in the human diet, is an activator of vascular smooth muscle L-type Ca^{2+} channels. This effect was rather specific, since the T-type Ca^{2+} channels were not affected by quercetin.

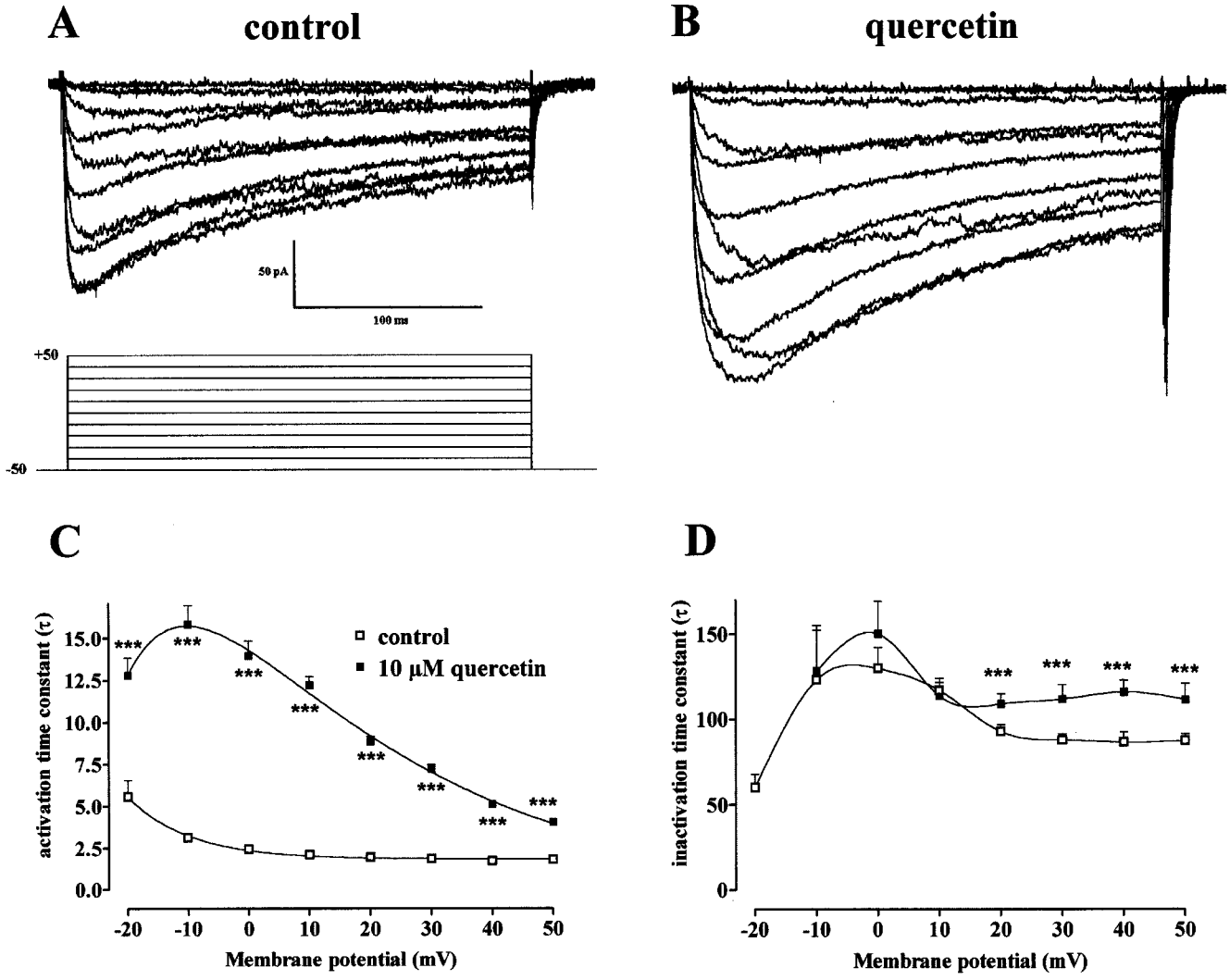

Figure 5 The effects of quercetin on both activation and inactivation kinetics. (A, B) Original recordings of conventional whole-cell $I_{Ca(L)}$ in rat tail artery myocytes elicited with 250-ms clamp pulses from a V_h of -50 mV to test potentials of -50 to 50 mV (see schematic diagram), measured in the absence (control) or presence of quercetin (10 μM). The time constant (τ) for activation (C) and inactivation (D), measured in the absence (control) or presence of quercetin (10 μM) is plotted against the membrane potentials. Each point represents the mean$\pm$s.e.mean of seven cells ($n=6$). ***$P<0.001$, Student's t-test for paired samples.

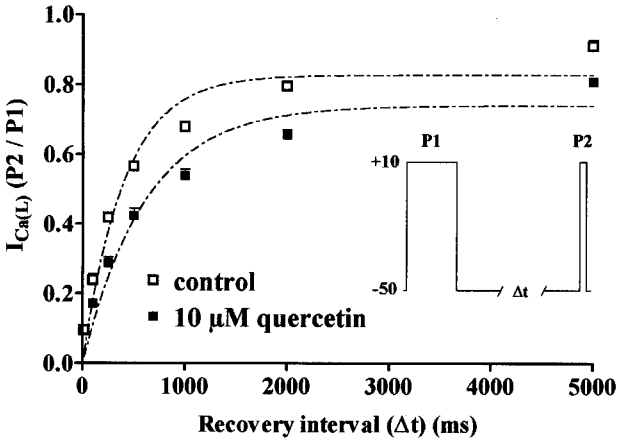

Figure 6 The effects of quercetin on recovery from inactivation. Recovery at V_h of -50 mV was obtained using a double-pulse protocol (see schematic inset). Two-sec clamp pulse to 10 mV from a V_h of -50 mV (pulse 1; P1) was followed by a second pulse (250 ms) to 10 mV from a V_h of -50 mV (pulse 2; P2). Delay between P1 and P2 (Δt) was variable in duration. Relative peak $I_{Ca(L)}$, measured in the absence (control) or presence of quercetin (10 μM), is plotted against recovery interval. Each point represents the mean $\pm$ s.e.mean of six cells ($n=5$).

Quercetin's activation of $I_{Ca(L)}$ contradicts its well-known vasodilatory effect (Formica & Regelson, 1995; Duarte *et al*., 1993), as it would be expected to cause contraction of the vascular musculature. As such, our observations suggest that the myorelaxing effect of quercetin in tissue preparations originates from its reaction with a second target, beyond the Ca^{2+} channel, which hierarchically prevails over the increase in the Ca^{2+} influx to be expected from $I_{Ca(L)}$ stimulation. This target might be represented by protein kinase C, since this enzyme, which plays a key role in maintenance of tonic contraction of vascular smooth muscle (Rasmussen *et al*., 1987), is inhibited by quercetin (Ferriola *et al*., 1989). This rather intriguing aspect of quercetin's effects on vascular smooth muscle preparations deserves further investigation.

In a recent paper, Summanen *et al*. (2001) demonstrated that quercetin increases I_{Ca} in clonal rat pituitary GH_4C_1 cells, possibly *via* a cyclic AMP-induced activation of protein kinase A. In vascular smooth muscle, however, both cyclic AMP and its related kinase, have been shown to inhibit L-type Ca^{2+} channels (Xiong & Sperelakis, 1995). Therefore it is unlikely that quercetin-induced activation of $I_{Ca(L)}$ observed here is the consequence of the increase in the intracellular levels of this cyclic nucleotide, caused by quercetin inhibition of cyclic AMP-phosphodiesterase (Nikaido *et al*., 1982). Furthermore, data obtained from amphotericin B-perforated cells, with minimal washout of the intracellular components, were comparable to those obtained from cells whose cytoplasm underwent extensive dialysis with the conventional method. It is therefore conceivable that quercetin stimulation of $I_{Ca(L)}$ is not mediated by diffusible intracellular factors but is rather the consequence of its direct interaction with the channel protein. Although many drugs are known to block L-type Ca^{2+} channels, only a few ligands predominantly activate them. Naturally occurring molecules such as heparin (Knaus *et al*., 1990) and certain animal toxins (Hamilton & Perez, 1987), have been shown to stimulate L-type Ca^{2+} channels in various mammalian tissues. Among the synthetic Ca^{2+} channel ligands, some dihydropyridine derivatives such as Bay K 8644 (Hess *et al*., 1984), CGP 28392 (Kokubun & Reuter, 1984), RS 30026 (Patmore *et al*., 1990) and (+) 1,4-dihydro-2,6-dimethyl-5-nitro-4-(benzofuran-5-yl)pyridine-3-carboxylate (Visentin *et al*., 1999) as well as FPL 64176, a benzoylpyrrole compound (Rampe & Lacerda, 1991), have been shown to possess predominantly agonist-like activity.

In this study, quercetin induced a peak current enhancement which was greatest at weak depolarization and became progressively smaller with increasing depolarization, and shifted the maximum of the $I_{Ca(L)}$-voltage relationship towards more negative potentials affecting neither E_{Ca} nor the threshold for $I_{Ca(L)}$. Thus, the effect of quercetin on the current–voltage relationship might be the consequence of the hyperpolarizing shift in the activation curve.

Quercetin shifted the voltage dependence of the inactivation and activation curves to more negative potentials and caused a significant increase in the slope of activation, which reflects a yield of more current for a given potential. These results indicate that quercetin may alter the voltage sensitivity of the channel. Furthermore, quercetin dramatically slowed Ca^{2+} channel activation kinetics over a wide range of membrane potentials, although this effect was more pronounced at weak depolarization. It is therefore likely that quercetin slows down the transition from the closed to the open state of the channel, or in some other way modifies its gating mechanisms. Additionally, quercetin significantly increased the inactivation time course only at voltages more positive than 10 mV and slowed down the time-dependence of recovery from inactivation. Both the shift in voltage dependence of inactivation and the increase of the time of recovery from inactivation suggest that quercetin may stabilize the inactivated state of the channel. Moreover, the inward tail currents decayed more slowly in the presence of quercetin, possibly reflecting a longer opening of the channel.

Taken together, all these elements indicate that quercetin shares several basic features with the properties of some Ca^{2+} channel activators such as Bay K 8644 and FPL 64176 (Wang *et al*., 1989; Rampe & Lacerda, 1991; Zheng *et al*., 1991). A direct comparison between quercetin and the other known activators as well as studies at the single channel level might provide the answer to the important question whether the effects of these compounds are additive.

Quercetin is a polyphenolic molecule with potent radical scavenging and antioxidant properties (Sgaragli *et al*., 1993). Several reports have demonstrated that vascular ion channels are potentially controlled by multiple redox-linked mechanisms (Wolin, 2000) and have raised the hypothesis of significant regional and/or tissue differences in redox-sensitive modulatory properties of L-type Ca^{2+} channels in the cardiovascular system (Campbell *et al*., 1996). Rutin, the glycosylated analogue of quercetin, which is an effective antioxidant as well as a radical scavenger (Rice-Evans *et al*., 1996), however, did not affect $I_{Ca(L)}$ in the same experimental model (unpublished observation). It therefore appears that quercetin did not modulate $I_{Ca(L)}$ in virtue of its antioxidant activity, once more supporting the view of a direct effect on the channel protein. Data have recently been collected to show how phenol derivatives exert interesting pharmacological effects on Ca^{2+} channels of smooth muscle cells. 3-*t*-

Butyl-4-hydroxyanisole (BHA) as well as 3,5-di-*t*-butyl-4-hydroxyanisole (DTBHA) have been demonstrated to inhibit $I_{Ca(L)}$ in cells isolated from guinea-pig gastric fundus musculature (Fusi *et al*., 2001a) and 2,5-di-*t*-butyl-1,4-benzohydroquinone has been shown to inhibit $I_{Ca(L)}$ in cells isolated from the rat tail main artery *via* superoxide anion generation (Fusi *et al*., 2001b). Therefore, phenol derivatives from natural or synthetic sources might represent a new class of Ca^{2+} channel modulators.

Although quercetin and dietary flavonoids have come into the focus of medicinal interest, data on their bioavailability after oral intake are scarce and contradictory (Day & Williamson, 2001). Quercetin aglycon and its metabolites have been detected in plasma of human volunteers in the range of 0.03–5 μM (Erlund *et al*., 2000; Olthof *et al*., 2000; Graefe *et al*., 2001). Furthermore, the rate of elimination of quercetin is relatively low and, therefore, repeated consumption of quercetin-containing foods might cause accumulation of quercetin in blood (Hollman *et al*., 1997) at levels extremely similar to those found to be active in the present study.

In conclusion, the present electrophysiological data point to quercetin as a vascular Ca^{2+} channel activator. Thus, this drug may be a useful pharmacological tool to further study the structure-function relationship and the gating mechanism of voltage-dependent Ca^{2+} channels.

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