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Effects of terpenoid phenol derivatives on calcium current in canine and human ventricular cardiomyocytes

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Abstract

Concentration-dependent (10–1000 μM) effects of terpenoid phenol derivatives were studied on L-type Ca^{2+} current in isolated canine and human ventricular cardiomyocytes using the whole-cell configuration of patch clamp technique. Carvacrol, thymol and eugenol suppressed peak Ca^{2+} current at +5 mV, having EC_{50} values and Hill coefficients of 98±11, 158±7 and 187±15 μM and 1.42±0.05, 2.96±0.43 and 1.6±0.1, respectively, in canine myocytes. Zingerone displayed a weak effect (estimated EC_{50}: 2±0.37 mM, Hill coefficient: 0.73±0.07), while vanillin and guaiacol failed to substantially modify Ca^{2+} current up to the concentration of 1 mM. In addition to tonic block, thymol and carvacrol, but not eugenol, evoked marked rate-dependent block at 2 Hz. Carvacrol and eugenol accelerated inactivation of Ca^{2+} current and caused leftward shift in the voltage dependence of steady-state inactivation without altering activation kinetics. Carvacrol, but not eugenol, increased the time constant of recovery from inactivation. These effects of carvacrol and eugenol developed rapidly and were largely reversible. In myocytes isolated from undiseased human hearts, the effect of carvacrol was similar to that observed in canine cells. It is concluded that suppression of cardiac Ca^{2+} currents by phenol derivatives is influenced by the substituent in the benzene ring, and the blocking effect of these drugs may involve interactions with the inactivation machinery of the channel.

Keywords: Cardiac cell; Ca^{2+} current; Phenol derivative; Electrophysiology; Myocyte, human

1. Introduction

Monoterpenoid phenol derivatives, like carvacrol [2-methyl-5-(1-methylethyl)phenol], thymol [5-methyl-2-(1-methylethyl)phenol] and eugenol (4-allyl-2-methoxyphenol), are widely used as general antiseptics in medical and dental practice, agriculture, cosmetics and food industry due to their potent fungicide, bactericide and antioxidant and anti-inflammatory properties (Aeschbach et al., 1994; Twetman et al., 1995; Shapiro and Guggenheim, 1995; Ogaard et al., 1997; Lee et al., 1997; Manou et al., 1998; Skold et al., 1998; Yucel-Lindberg et al., 1999). Zingerone [(4-hydroxy-3-methoxyphenyl)ethyl] and vanillin (4-hydroxy-3-methoxybenzaldehyde) are major components of several spice plants; in addition, they are used as potent vanilloid receptor agonists (Sterner and Szallasi, 1999). Guaiacol (2-methoxyphenol) is also used in medical practice as an expectorant. The chemical structures of the studied phenol derivatives are shown in Fig. 1.

In spite of the widespread human application of these compounds (in foods, drinks, medicines and mouthwash), little is known about their effects in mammalian cardiac tissues. K^+ currents are suppressed by zingerone (Castle, 1992), eugenol (Sensch et al., 2000) and thymol (Magyar et al., 2002) in various types of mammalian cardiomyocytes. In the recent paper of Magyar et al. (2002), thymol was shown also to inhibit L-type Ca^{2+} current (I_{Ca}) in canine and human ventricular cells, and kinetic properties of this effect of thymol were analyzed. The aim of the present work, therefore, was to study and compare the effects of these monoterpenoid phenol derivatives on I_{Ca} in isolated canine and healthy human ventricular cardiomyocytes in order to

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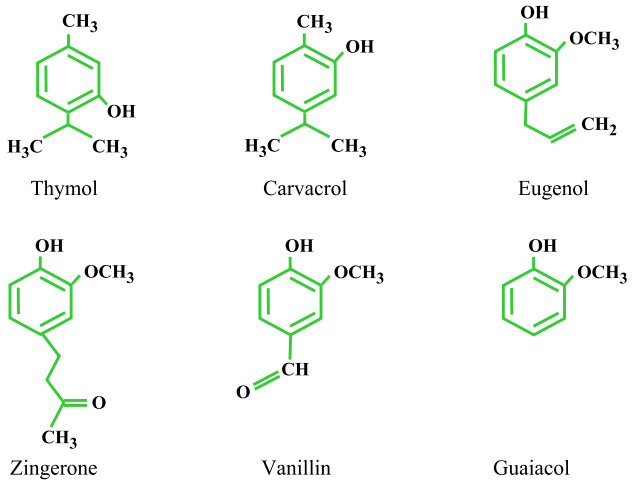

Fig. 1. Chemical structure of the phenol derivatives studied.

reveal their mechanism of action and to predict their possible cardiac effects in human.

2. Materials and methods

2.1. Cell isolation

Isolated canine ventricular myocytes were obtained from hearts of adult mongrel dogs using the segment perfusion technique (Magyar et al., 2000a). Briefly, the animals (10–20 kg) were anesthetized with intravenous injection of 10 mg/kg ketamine hydrochloride (Calypsolvet)+1 mg/kg xylazine hydrochloride (Rometar). The hearts were rapidly removed from the chest and one of the coronary arteries (usually the left anterior descendent branch) was cannulated and perfused with Ca^{2+}-free JMM solution (Minimum Essential Medium Eagle Joklik Modification for Suspension Culture, Type M-0518, SIGMA) for 5 min in order to remove Ca^{2+} from the tissues. The pH of this solution was adjusted to 7.2 using $NaHCO_3$ when gassed with carbogen at 37 °C. Dispersion of the cells was performed during a 30-min perfusion with JMM solution containing 20 μM $CaCl_2$ and 1 g/l collagenase (Worthington, Type CLS1). The enzyme was removed from the tissues during a 5-min additional perfusion with enzyme-free JMM solution. Dispersed ventricular cells were suspended in the same solution, filtered, sedimented and then transferred to MEM solution (Minimum Essential Medium Eagle, M-0643, SIGMA) containing 2.5 mM $CaCl_2$ and having pH adjusted to 7.35. The myocytes were rod-shaped and showed clear striation when the external Ca^{2+} was restored following cell isolation. Cells were stored in MEM solution overnight at 15 °C.

Human ventricular cells were prepared from hearts of general organ donor patients undergoing aortic valve transplantation surgery. The procedures used on human tissues have been approved by a local ethical committee and comply with the current laws of Hungary. The myocytes were isolated using a recently developed enzymatic dissociation procedure (Magyar et al., 2000b). After explantation and removal of the valves, hearts were transported into the laboratory in cold cardioplegic solution. A portion of the left ventricular wall was excised together with its arterial branch and was mounted on a modified Langendorff apparatus, where it was perfused through the left anterior descending coronary artery according to the following sequence: (1) modified Tyrode solution (containing NaCl, 135; KCl, 4.7; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $MgSO_4$, 1.2; HEPES, 10; $NaHCO_3$, 4.4; glucose 10 mM; pH=7.2) for 10 min, (2) Ca^{2+}-free modified Tyrode for 10 min and (3) Ca^{2+}-free modified Tyrode containing collagenase (660 mg/l, type I, Sigma), elastase (45 mg/l, type III, Sigma), taurine (50 mM) and bovine albumin (2 g/l, fraction V, fatty acid free, Sigma) for 45 min. (4) After this step of enzymatic digestion, the solution was supplemented with protease (120 mg/l, type XIV, Sigma) for further 40–60 min. Portion of the left ventricular wall, which was clearly digested by the enzymes, was cut into small pieces and either stored in KB medium (*Kraftbrühe*, Isenberg and Klöckner, 1982) or equilibrated for 15 min in modified Tyrode containing 1.25 mM $CaCl_2$ and 50 mM taurine. Single myocytes were obtained from the tissue chunks after gentle agitation. During the entire isolation procedure, the solutions were oxygenated (100% O_2) and the temperature was maintained at 37 °C. The cells were allowed to sediment for 10 min, then the supernatant was decanted and replaced by fresh solution. This procedure was repeated three times. The cells in KB medium were stored at 4 °C, while those stored in modified Tyrode solution were maintained at 12–14 °C before use.

2.2. Voltage clamp

I_{Ca} was recorded from Ca^{2+}-tolerant ventricular cells superfused with oxygenated Tyrode solution at 37 °C. The bathing medium contained NaCl 140, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, Na_2HPO_4 0.35, HEPES 5, glucose 10, 4-aminopyridine 3 mM, at pH 7.4. Suction pipettes, fabricated from borosilicate glass, had tip resistance of 2 MΩ after filling with pipette solution composed of KCl 110, KOH 40, HEPES 5, EGTA 10, TEACl 20, K-ATP 3 and GTP 0.25 mM (pH=7.2). The current was recorded with an Axopatch-200B amplifier (Axon Instruments) using the whole-cell configuration of the patch clamp technique (Hamill et al., 1981). After establishing high (1–10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5-V electrical pulses for 1–5 ms. After this step, the intracellular solution was allowed to equilibrate with the pipette solution for a period of 5–10 min before starting the measurement. I_{Ca} was normalized to cell capacitance, determined in each cell using short (25 ms) hyperpolarizing pulses from 0 to −10 mV. The series resistance was

typically 4–8 MΩ before compensation (usually 50–80%). Experiments were discarded when the series resistance was high or substantially increasing during the measurement. Outputs from the clamp amplifier were digitized at 10 kHz using an A/D converter (Digidata-1200, Axon Instruments) under software control (pClamp 6.0, Axon Instruments). The applied experimental protocols are described in the results section where appropriate.

2.3. Drug application

All experimentally applied phenol derivatives were suspended in dimethyl sulfoxide, so as to produce stock solutions having 100 mM drug concentration. This solution was stored in refrigerator and was freshly diluted with Tyrode solution before the experiment to reach the concentration required. The maximal drug concentration applied during the cumulative concentration–response experiments was 1 mM. This solution contained 1% dimethyl sulfoxide, which was experimentally shown to have no effect on the Ca^{2+} current. Before taking control records, I_{Ca} was continuously monitored until its amplitude stabilized. In the case of progressive rundown, the measurement was discarded. Myocytes were superfused with drugs for 2 min and washout lasted for 5 min. In experiments studying cumulative concentration-dependent effects, each concentration of drug was applied for 2 min. All values presented are arithmetic means±S.E.M. Student's *t*-test for paired data was applied following analysis of variance (ANOVA) to determine statistical significance. Differences were considered significant when the *P* value was less than 0.05.

The entire investigation complies with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and the principles outlined in the *Declaration of Helsinki*. The experimental protocol was approved by the Institutional Ethics Committee.

3. Results

3.1. Concentration-dependent effect of phenol derivatives on I_{Ca} in canine myocytes

Peak I_{Ca} was measured at a rate of 0.2 Hz using depolarizing voltage pulses of 400-ms duration, clamped from the holding potential of −40 mV to the test potential of +5 mV. K^+ currents were blocked by the externally applied 4-aminopyridine and internally applied TEACl. Stability of I_{Ca} was monitored at least for 5 min before cumulative application of drugs (from 10 to 1000 μM, each concentration for 2 min). As shown in Fig. 2., 300 μM thymol, carvacrol and eugenol markedly suppressed peak I_{Ca} at +5 mV; zingerone displayed only a weak effect at this concentration, while vanillin and guaiacol failed to decrease I_{Ca}. Inhibition of I_{Ca} developed rapidly (within 1 min) and was largely reversible upon washout. The cumulative concentration–response curves obtained with the phenol derivatives are displayed in Fig. 3. Fitting data to the Hill equation yielded EC_{50} values and Hill coefficients of 98±11, 158±7 and 187±15 μM and 1.42±0.05, 2.96±0.43 and 1.6±0.1, respectively, for carvacrol, thymol and eugenol. A substantially higher EC_{50} value (2±0.37 mM) was estimated for zingerone with Hill coefficient of 0.73±0.07.

In addition to the tonic block measured at 0.2 Hz, trains, composed of 20 short (50 ms) depolarizing pulses to +5 mV,

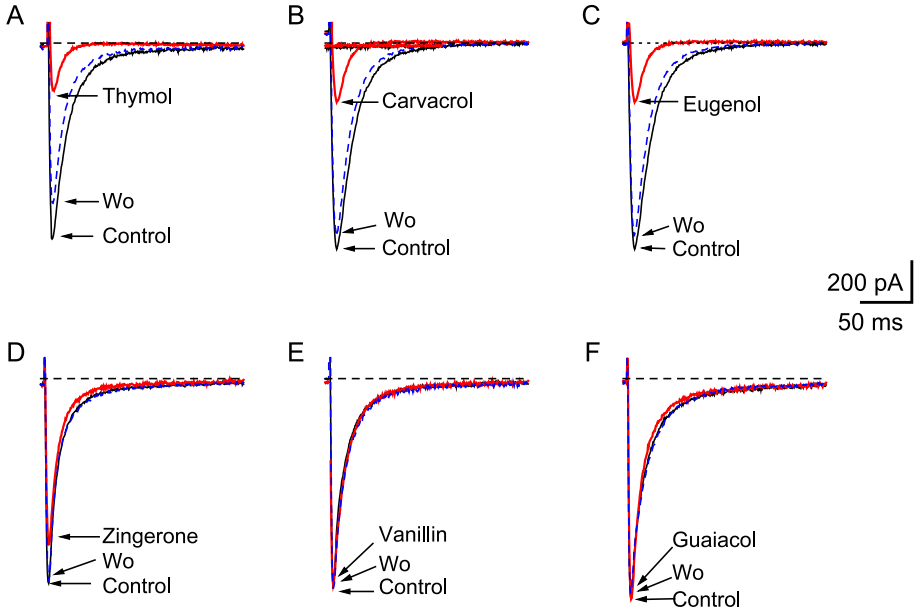

Fig. 2. Superimposed Ca^{2+} current records, taken at +5 mV, showing the reversible effects of phenol derivatives (300 μM each) in canine ventricular cells.

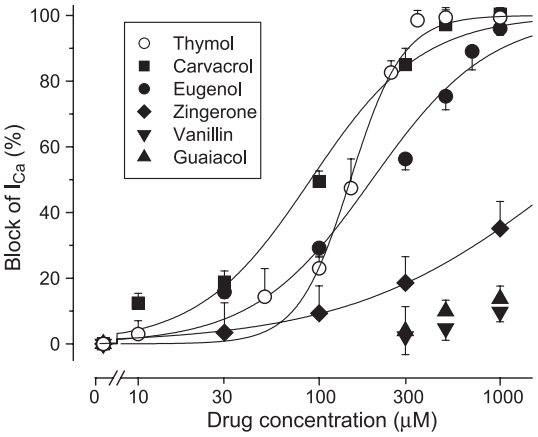

Fig. 3. Cumulative concentration-dependent effects of thymol (n=4), carvacrol (n=7), eugenol (n=6), zingerone (n=9), vanillin (n=8) and guaiacol (n=8) on the peak Ca^{2+} current, measured at +5 mV. The solid line was obtained by fitting data to the Hill equation. Symbols and bars represent mean values±S.E.M.

were applied at 2 Hz in order to estimate rate-dependent block. In these experiments, the cells were initially stimulated at a constant frequency of 0.2 Hz until reaching steady-state values for I_{Ca}, then a train of pulses was applied at a rate of 2 Hz. Finally, the stimulation frequency returned to 0.2 Hz. The peak value of I_{Ca} decreased continuously during the train; the magnitude of this reduction was expressed as a difference between the amplitudes of I_{Ca} evoked by the 1st and the 20th pulse of the train (I_1 and I_{20}, respectively) normalized to I_1. On returning to the lower, 0.2-Hz frequency, I_{Ca} recovered rapidly (within 20 s). The train-induced decay of I_{Ca} was 20.1±2.3% in Tyrode solution (n=12), which increased to 33.3±3.2%, 46.7±2.1% and 23.6±0.6%, respectively, in the presence of 100 μM thymol, carvacrol and eugenol (P<0.05, n=4 for each). The rate-dependent block induced by the compound was estimated as a difference between the $(I_1-I_2)/I_1$ ratios measured in the presence and absence of the drug: values of 13.2%, 26.6% and 3.5% were obtained, respectively, with 100 μM thymol, carvacrol and eugenol. For comparison, the tonic block induced by the same concentration of the respective drugs at 0.2 Hz was 23±4%, 49±3% and 30±3%.

3.2. Effects of carvacrol, eugenol and zingerone on the kinetic properties of I_{Ca} in canine myocytes

Since vanillin and guaiacol had negligible effect on I_{Ca}, and the effects of thymol have been previously

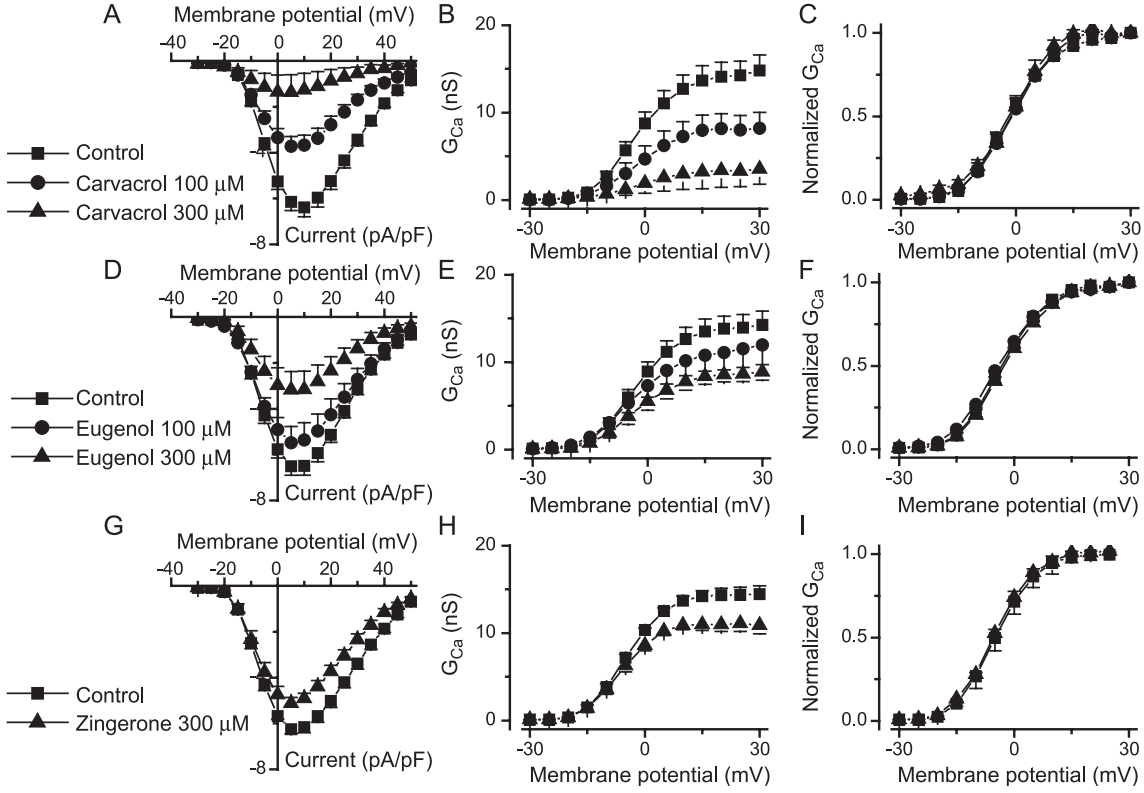

Fig. 4. Effect of carvacrol (A–C), eugenol (D–F) and zingerone (G–I) on voltage-dependent activation of the Ca^{2+} current (n=5 for each). A, D and G: Current–voltage relationships obtained for peak Ca^{2+} current in canine myocytes in control and in the presence of 100 and 300 μM carvacrol and eugenol or 300 μM zingerone, respectively. B, E and H: Ca^{2+} conductance (G_{Ca}) calculated from the $I-V$ curves in control and in the presence of the respective drugs. C, F and I: $G_{Ca}-V_m$ relationships obtained from the experiments above, but G_{Ca} values were normalized to G_{Ca} obtained at +30 mV, and the results were fitted to a two-state Boltzmann function (solid curves).

characterized (Magyar et al., 2002), the properties of I_{Ca} were analyzed only in the presence of carvacrol, eugenol and zingerone. Current–voltage relations for I_{Ca} were obtained by applying a series of 400-ms-long test pulses increasing up to +50 mV in 5-mV steps in absence and presence of the drug, and peak values of I_{Ca} were plotted against their respective test potentials. The maximum value of peak current was obtained around +5 mV, and no shift in the current–voltage relationship was observed after application of 100 and 300 μM carvacrol and eugenol or 300 μM zingerone (Fig. 4A, D, and G). Ca^{2+} conductance (G_{Ca}) was calculated at each membrane potential by dividing the peak current by its driving force (the difference between the applied test potential and the reversal potential for I_{Ca}, estimated to be +55 mV). As shown in Fig. 4B, E, and H, the maximum Ca conductance was significantly diminished by 300 μM carvacrol, eugenol and zingerone (from 14.4±0.15 to 3.5±0.03, from 14±0.12 to 8.7±0.09 and from 14.5±0.1 to 10.9±0.1 nS, respectively, n=5 for each). When G_{Ca} values of both curves were normalized to the respective G_{Ca} obtained at +30 mV, the G_{Ca}–V_m relationships were identical indicating that voltage dependence of activation of I_{Ca} is not affected by carvacrol, eugenol and zingerone (Figs. 4C, F, and I).

In contrast to the unchanged voltage dependence of activation, inactivation kinetics were seriously altered in the presence of carvacrol and eugenol in a reversible manner. In order to study the voltage dependence of steady-state inactivation of I_{Ca}, test depolarizations to +5 mV were preceded by a set of prepulses clamped to various voltages between −55 and +15 mV for 500 ms. Peak currents measured after these prepulses were normalized to the peak current measured after the −55-mV prepulse and plotted against the respective prepulse potential. The data were fitted to the two-state Boltzmann function (Fig. 5). Superfusion of the cells with 100 and 300 μM carvacrol or eugenol shifted the midpoint potentials by 7.2±0.4 and 13.8±1.26 mV (P<0.001, n=5), or 3.84±0.5 and 6.05±1.18 mV (P<0.001, n=5), respectively, from the control value of −20.34±0.75 mV towards more negative potentials without significant changes in the

Table 1
Effect of 300 μM carvacrol, eugenol and zingerone on inactivation kinetics of Ca^{2+} current in canine ventricular myocytes

	τ_1 (ms)	A_1 (pA/pF)	τ_2 (ms)	A_2 (pA/pF)
Control	13.6±0.6	−4.9±0.54	70.6±2.3	−0.95±0.2
Carvacrol	7.9±0.7[c]	−1±0.21[b]	77.1±7.1	−0.12±0.15[b]
Washout	13.4±1.3	−3.7±0.31[a]	68.5±5.5	−0.41±0.12[a]
Control	15.2±1	−4.3±0.19	68.9±1.6	−0.83±0.12
Eugenol	12.8±0.5[a]	−2.4±0.18[c]	63.9±2.6	−0.45±0.05[b]
Washout	14.4±2.6	−3.4±0.13[a]	63.5±4.9	−0.55±0.02[a]
Control	12.7±0.7	−4.7±0.64	75.3±5	−1.02±0.22
Zingerone	11.5±0.6[b]	−4.1±0.38	66.7±5.9	−0.64±0.11[a]
Washout	12.4±0.8	−4.3±0.42	86.3±3.4	−0.99±0.07

The decay of I_{Ca} was fitted as a sum of two exponential components, each characterized by a time constant (τ) and a relative amplitude (A). Index numbers 1 and 2 refer to the fast and slow components, respectively. Mean±S.E.M. are given, superscripts denote significant changes from control values (P<0.05[a], P<0.01[b], P<0.001[c]); n=5 for carvacrol and eugenol; n=7 for zingerone.

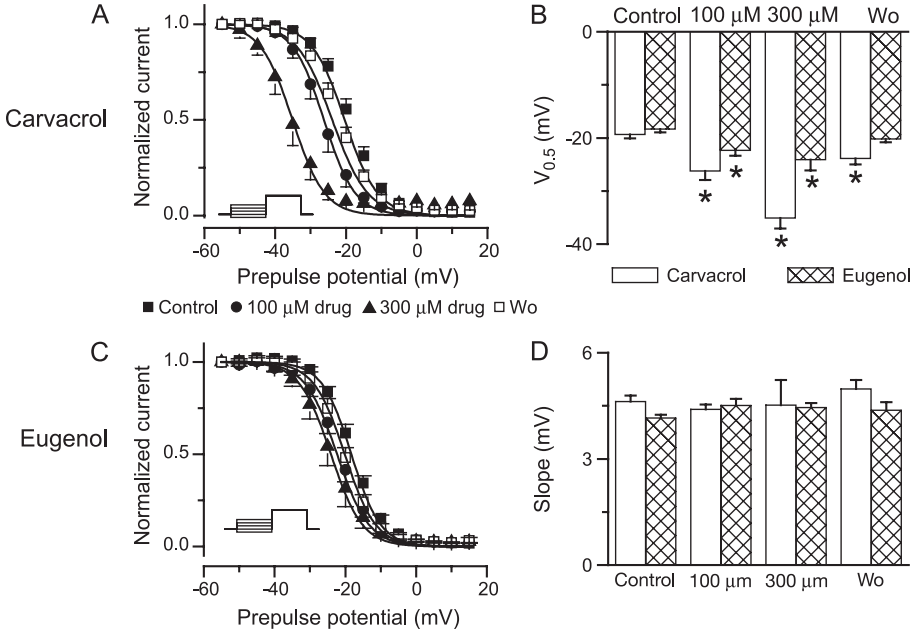

Fig. 5. Effects of carvacrol and eugenol on the steady-state inactivation of Ca^{2+} current. A and C: Voltage dependence of steady-state inactivation determined using paired-pulse protocol in the presence and absence of carvacrol and eugenol (n=5 for each). Solid curves were obtained by fitting data to a two-state Boltzmann model. Estimated midpoint potentials ($V_{0.5}$) and slope factors are presented in the right panels (B and D, respectively). Symbols and bars represent mean values±S.E.M.; asterisks denote significant (P<0.05) changes compared to control.

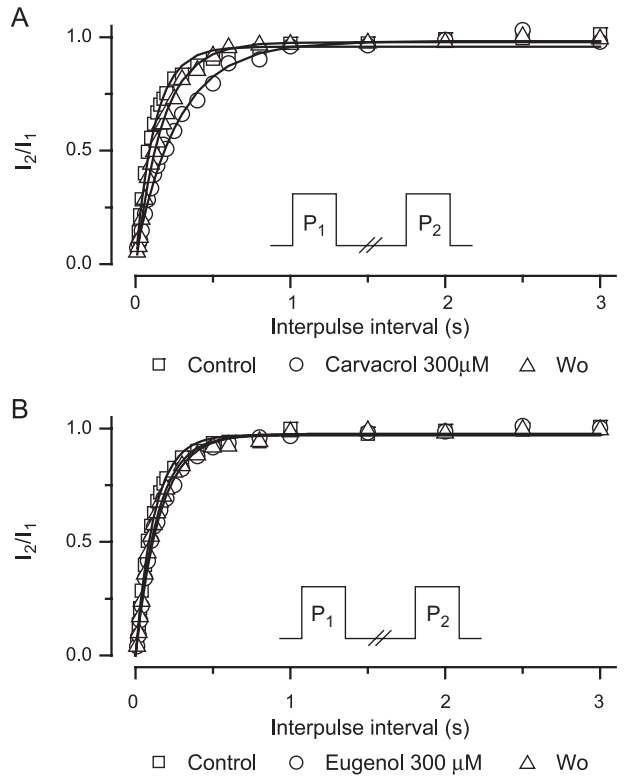

Fig. 6. Time course of recovery from inactivation measured with twin-pulse protocols in the absence and presence of 300 μM carvacrol (A, n=6) or eugenol (B, n=5). The curves were obtained by fitting data to a single exponential function.

slope factor (4.62±0.17 mV in control). Zingerone (300 μM) failed to alter the voltage dependence of steady-state inactivation.

In addition to increase steady-state inactivation, carvacrol, eugenol and zingerone accelerated the time-dependent decay of I_{Ca}, as can be seen in the analogue records of Fig. 2. The decay of I_{Ca} followed bi-exponential kinetics at +5 mV, characterized as a sum of a fast and a slow component in control. Application of 300 μM carvacrol, eugenol or zingerone reduced the relative amplitude of both components of inactivation and decreased the time constant of the fast component, while the time constant of the slow component remained unaffected. These time constants and corresponding relative amplitudes are summarized in Table 1.

Time course of recovery from inactivation was studied using a twin-pulse protocol. Two 400-ms-long depolarizations to +5 mV were separated by interpulse intervals having variable durations. Peak I_{Ca} amplitude measured by the second pulse was normalized to that measured by the first pulse, and their ratio (I_2/I_1) was plotted against the interpulse interval. The curves, shown in Fig. 6, were obtained by fitting the results to a single exponential function. Carvacrol (300 μM) increased the time constant for recovery from inactivation significantly (from 122±8 to 231±26 ms, P<0.05, n=6), whereas the same concentrations of eugenol and zingerone (not shown) failed to modify recovery kinetics.

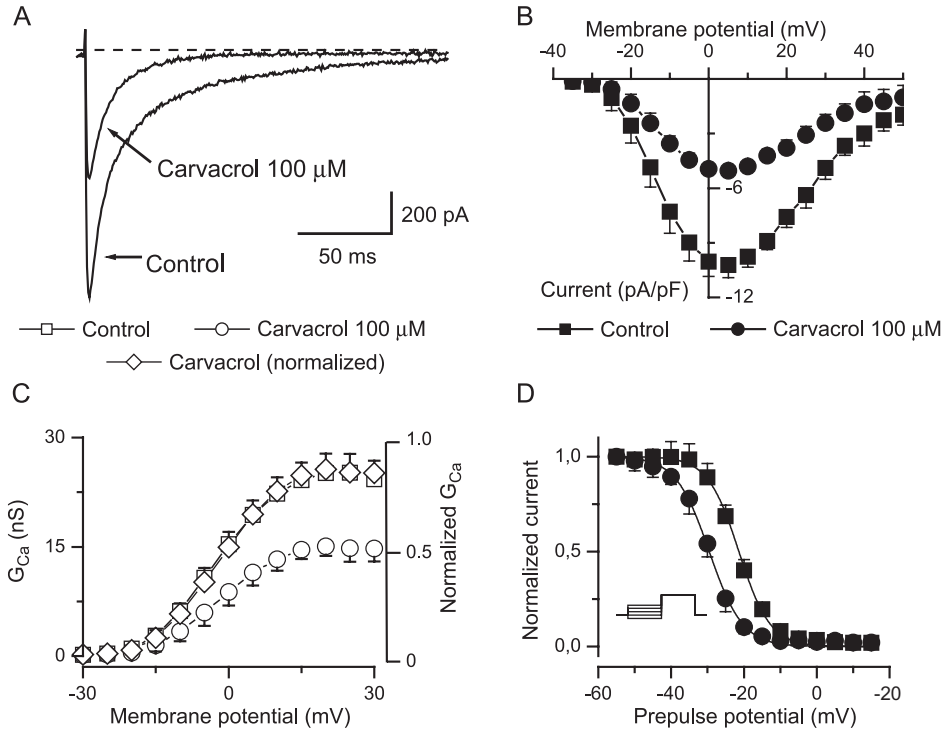

Fig. 7. Effect of 100 μM carvacrol on the Ca^{2+} current in ventricular myocytes (n=4) isolated from undiseased human hearts. (A) Superimposed I_{Ca} records measured at +5 mV. (B) Current–voltage relationships. (C) Normalized G_{Ca}–V_m relationships. (D) Voltage dependence of steady-state inactivation. Measurements were performed as described earlier for canine cells.

3.3. Effect of carvacrol on Ca^{2+} current in undiseased human ventricular cells

Effects of 100 μM carvacrol in the human cells (Fig. 7, n=4) were similar to those obtained in canine myocytes. Carvacrol suppressed the peak value of I_{Ca} at each test potential studied (from −10±0.41 to −4.9±0.03 pA/pF at +5 mV, P<0.05) without changing the shape of the $I-V$ relationship and the normalized $G_{Ca}-V_m$ curve (midpoint potential=−2.9±0.28 versus −2.2±0.25 mV, slope factor=6.1±0.24 versus 6.0±0.21 mV, N.S.). Effect on the voltage dependence of steady-state inactivation was also similar to those seen in the canine cells: the midpoint of the Boltzmann function was shifted from −21.4±0.56 to −29.5±0.44 mV (8.1±0.6 mV leftward shift, P<0.05), while the slope factor remained unaltered (4.3±0.28 versus 4.6±0.24 mV, N.S.). Similar to results obtained in canine cells, carvacrol (100 μM) reduced the amplitude of both components of inactivation (from −6.4±0.51 to −4.15±0.17 pA/pF and from −2.94±0.3 to −0.65±0.03 pA/pF for the faster and slower component, respectively, P<0.05 for both) and decreased the faster time constant of inactivation (from 13.3±0.91 to 10.1±0.18 ms, P<0.05). The slower time constant was also decreased (from 69±5 to 57±7 ms); however, this change was not significant statistically. The time constant of recovery from inactivation was not significantly altered by 100 μM carvacrol in the human cells (99±8.7 versus 119±16 ms, N.S.).

4. Discussion

The physicochemical properties of an aromatic molecule depend primarily on the substituents of the benzene ring which can strongly influence distribution of the electrons along the molecule: hydrophobic tail substituents are known to promote lipid solubility. In our experiments, the strongly hydrophobic (water-insoluble) terpenoid carvacrol and eugenol—similarly to thymol—suppressed L-type Ca^{2+} current in a concentration-dependent manner, whereas guaiacol and vanillin, which are less hydrophobic (water-soluble) compounds, had no effect on the current. These results suggest that terpenoid phenol derivatives, due to their hydrophobic character, may exert their effects, at least in part, on the lipid–protein interface by altering the local environment of ion channels. The fractional (not integer) values of the Hill coefficients obtained for carvacrol, eugenol and zingerone may give support to this explanation. However, in the case of thymol, the Hill coefficient was close to 3, suggesting strong positive cooperation between discrete binding sites involved or, alternatively, contribution of more than one mechanism to the block. It is possible, therefore, that in addition to the postulated lipid interactions, thymol may bind to and thus modify the channel protein directly. Surprisingly—similarly to the results of Sensch et al. (2000)—EC_{50} values failed to fully correlate with the lipophilic nature of the molecules. Since the longer tail and the other substituents of the benzene ring may decrease the dissociation of proton from the hydroxyl group, binding of the molecule to the channel can consequently decrease (Erdélyi, 1999). Indeed, among the hydrophobic compounds, zingerone had the longest tail and the weakest blocking potency. The size of tail decreased from zingerone through eugenol to thymol and carvacrol; accordingly, the Ca^{2+} channel blocking potency of the molecules increased. The distance between the hydroxyl group and the tail substituent is greater in the case of carvacrol than thymol, explaining why the suppressive effect of carvacrol was stronger than that of thymol.

Inhibitory action of carvacrol and eugenol on the Ca^{2+} current, similarly to earlier observations with thymol (Magyar et al., 2002), included reduction of the peak current, acceleration of the current decay and leftward shift in the voltage dependence of steady-state inactivation; however, activation kinetics were not affected. In addition to these changes obtained at low frequency of stimulation (0.2 Hz), marked rate-dependent block was observed at 2 Hz with carvacrol and thymol, but not with eugenol. Taking into account that carvacrol, but not eugenol, retarded recovery from inactivation, and the leftward shift obtained in the voltage dependence of steady-state inactivation was much greater with carvacrol than eugenol, it seems likely that the inhibitory action of these terpenoids may be related—at least in part—to their interaction with the inactivation machinery of the channel. Based on these results, the blocking effect of the terpenoid phenol derivatives on cardiac Ca^{2+} current resembles that of many conventional Ca^{2+}-entry blocker agents, like verapamil or nifedipine. In addition, their effect is likely not restricted to cardiac tissues since these drugs were reported to effectively suppress Ca^{2+} current in neuronal membranes as well (Győri et al., 1991; Erdélyi, 1992; Szabadics and Erdélyi, 2000). Although thymol was shown to induce Ca^{2+} release from internal stores in neural (Kostyuk et al., 1991), smooth muscle (Hisayama and Takanayagi, 1986), skeletal muscle (Szentesi et al., 2001) and cardiac tissues (own unpublished results), this effect might not substantially modify interpretation of the present results due to the presence of 10 mM EGTA in our pipette solution.

It is important to emphasize that the effect of carvacrol on the Ca^{2+} current was comparable in canine and healthy human ventricular cells. For instance, 100 μM carvacrol induced a 49% block of peak Ca^{2+} current at +5 mV in the canine and 51% inhibition in the human myocytes. Similarly, the negative shift caused by carvacrol in the steady-state inactivation curve was 7.2±0.4 mV in canine and 8.1±0.6 mV in human cells. Based on these numbers, one may conclude that the mechanism of action of carvacrol—and probably of the other related compounds—on the Ca^{2+} channels is essentially similar in dog and human. Regarding the toxicological relevance of our results, some predictions

can be made. Although no data on possible plasma concentrations are available in the literature, it can be calculated on the example of thymol. Several types of mouthwash contain 1% thymol in 200 ml of fluid. Ingestion of this solution (accidentally by children or intentionally by adults in case of suicide) will build up approximately 300 μM concentration in the whole body fluid (supposing equal distribution in extracellular and intracellular body water compartments). In addition to suppression of the Ca^{2+} current, these concentrations of thymol (Magyar et al., 2002), carvacrol and eugenol (own unpublished results) caused variable but pronounced modifications in the configuration of the action potential including reduction of its amplitude and velocity of depolarization and shortening or lengthening of its duration. Based on the results above, cardiac arrhythmias and heart failure can be anticipated in case of intoxication in humans.

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