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ORIGINAL ARTICLE

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Different effects of endothelin-1 on calcium and potassium currents in canine ventricular cells

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Abstract Effects of endothelin-1 (ET-1) on the L-type calcium current (I_{Ca}) and delayed rectifier potassium current (I_K) were studied in isolated canine ventricular cardiomyocytes using the whole-cell configuration of the patch-clamp technique. ET-1 (8 nM) was applied in three experimental arrangements: untreated cells, in the presence of 50 nM isoproterenol, and in the presence of 250 μM 8-bromo-cAMP. In untreated cells, ET-1 significantly decreased the peak amplitude of I_{Ca} by 32.3±4.8% at +5 mV (P<0.05) without changing activation or inactivation characteristics of I_{Ca}. ET-1 had no effect on the amplitude of I_K, I_{to} (transient outward current) or I_{K1} (inward rectifier K current) in untreated cells; however, the time course of recovery from inactivation of I_{to} was significantly increased by ET-1 (from 26.5±4.6 ms to 59.5± 1.8 ms, P<0.05). Amplitude and time course of intracellular calcium transients, recorded in voltage-clamped cells previously loaded with the fluorescent calcium indicator dye Fura-2, were not affected by ET-1. ET-1 had no effect on force of contraction in canine ventricular trabeculae.

Isoproterenol increased the amplitude of I_{Ca} to 263± 29% of control. ET-1 reduced I_{Ca} also in isoproterenol-treated cells by 17.8±2% (P<0.05); this inhibition was significantly less than obtained in untreated cells. I_K was increased by isoproterenol to 213±18% of control. This effect of isoproterenol on I_K was reduced by 31.8±4.8% if the cells were pretreated with ET-1. Similarly, in isoproterenol-treated cells ET-1 decreased I_K by 16.2±1.5% (P<0.05). Maximal activation of protein kinase A (PKA) was achieved by application of 8-bromo-cAMP in the pipette solution. In the presence of 8-bromo-cAMP ET-1 failed to alter I_{Ca} or I_K. It was concluded that differences in effects of ET-1 on I_{Ca} and I_K may be related to differences in cAMP sensitivity of the currents.

Keywords Cardiac cells · Action potential · Endothelin · Calcium current · Potassium currents · Signal transduction · cAMP

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Introduction

Endothelins are recently described vasoactive peptides released from vascular and endocardial endothelium. They represent a group of three isopeptides, from which endothelin-1 (ET-1) is an extremely potent vasoconstrictor (Yanagisawa et al. 1988; Haynes and Webb 1993). Endothelin receptors have been detected in various cardiac membranes (Moody et al. 1990) and there is now an increasing mass of evidence suggesting that ET-1 may be an important factor in cardiovascular regulation under physiological and various pathological conditions (Haynes and Webb 1993).

Effects of ET-1 on cardiac cells are controversial, strongly depending on the experimental conditions, the origin and developmental state of the preparation studied. ET-1 was shown to have positive inotropic action in several mammalian cardiac preparations (Moravecz et al. 1989; Kelly et al. 1990); however, according to other investigators the effects of ET-1 rather resembled those of acetylcholine (Kim 1991; Zhu et al. 1997). Indeed, ET-1 caused hyperpolarization, shortening of action potentials and reduction in heart rate due to inhibition of I_{Ca} and activation of K currents (Ono et al. 1994; Delpech et al. 1995; Xie et al. 1996).

There are marked interspecies differences observed with the actions of ET-1 on various cardiac ion currents; in addition, inconsistent results were reported even in the same species. For example, action potentials were lengthened by ET-1 in rat ventricular myocytes (Damron et al. 1993), but shortened in guinea pig and rabbit atrial cells

(Ono et al. 1994). I_{Ca} was decreased (Xie et al. 1996) or increased (Lu et al. 1995) by ET-1 in guinea pig ventricular cells. In rabbit ventricular myocytes I_{Ca} was also decreased by ET-1; however, I_{Ca} increased if ET-1 was applied in GTP-dialysed cells (Lauer et al. 1992). In atrial myocytes of rabbit and guinea pig I_{Ca} was reduced by ET-1 (Ono et al. 1994; Delpech et al. 1995). This effect was cAMP-dependent in rabbit, but cAMP-independent in guinea pig. In embryonic chick and human ventricular myocytes ET-1 enhanced I_{Ca} (Bkaily et al. 1995). Regarding K currents, I_K was inhibited by ET-1 in adult rat myocytes (Damron et al. 1993), was enhanced in adult guinea pig cells (Lu et al. 1995), or left unaffected in embryonic chick and human myocytes (Bkaily et al. 1995). ET-1 increased the inward rectifier K current in atrial cells of rat, rabbit and guinea pig (Kim 1991; Ono et al. 1994), but suppressed the acetylcholine-activated K current in rabbit atrial myocytes (Spiers et al. 1996).

ET-1 was also shown to inhibit the PKA-dependent Cl^- current (James et al. 1994) in rabbit and guinea pig myocytes. These effects of ET-1 were accompanied with fall in intracellular cAMP level (Hilal-Dandan et al. 1992; Xie et al. 1996) which was sensitive to pertussis toxin (Ono et al. 1994). Since the inhibitory actions of ET-1 were greatly enhanced when studied following isoproterenol treatment, the general picture emerged that the main mechanism for action of ET-1 is to inhibit the adenylate cyclase enzyme via the G-protein-coupled ET_A receptors (Haber and Lee 1994; James et al. 1994; Xie et al. 1996).

Among the currently studied mammalian ventricular preparations the electrophysiological properties of canine ventricular myocytes – including distribution of various ionic currents – resemble best those of human cells. Due to the limited access to undiseased human ventricular tissues, canine myocyte might be expected to be a suitable model for studying the actions of ET-1 in man. The aim of the present work, therefore, was to study the effects of ET-1 on two important cAMP-dependent currents, I_{Ca} and I_K, in adult canine ventricular myocytes, and to investigate the mechanism of action of ET-1 in this species.

Materials and methods

Cell isolation. Single canine ventricular myocytes were obtained from hearts of adult mongrel dogs using the segment perfusion technique. The animals (10–20 kg) were anesthetized with i.v. injection of 10 mg/kg ketamine hydrochloride (Calypsolvet) + 1 mg/kg xylazine hydrochloride (Rometar). After opening the chest the heart was rapidly removed and the left anterior descending coronary artery was perfused using a Langendorff apparatus. During the initial 5 min of perfusion Ca^{2+}-free JMM solution (Minimum Essential Medium Eagle, Joklik modification; Sigma, product no. M-0518), supplemented with taurine (2.5 g/l), pyruvic acid (175 mg/l), ribose (750 mg/l), allopurinol (13.5 mg/l) and NaH_2PO_4 (200 mg/l), was used to remove Ca^{2+} and blood from the tissue. After addition of $NaHCO_3$ (1.3 g/l), the pH of this perfusate was 7.0±0.05 when gassed with carbogene. Cell dispersion was performed for 30 min in the same solution containing also collagenase (660 mg/l; Worthington Cls-1), bovine albumine (2 g/l) and $CaCl_2$ (50 μM). During the entire isolation procedure the solutions were gassed with carbogene and the temperature was maintained at 37°C. The cells were rod shaped and showed clear striation when the external calcium was restored. Before use, the cells were stored overnight at 14°C in modified JMM solution (pH=7.4).

Voltage clamp. Experiments were performed in Ca^{2+}-tolerant myocytes at 37°C. The cells were rod shaped and maintained clear cross-striation following exposure to oxygenated Tyrode solution containing (in mM) NaCl, 140; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.2; Na_2HPO_4, 0.35; HEPES, 5 and glucose, 10 at pH of 7.4. This solution was supplemented with either 0.25 mM $CdCl_2$ or 3 mM 4-aminopyridine when potassium or calcium currents, respectively, were measured. Suction pipettes, fabricated from borosilicate glass, had tip resistance of 2 MΩ after filling with pipette solution containing (in mM) K-aspartate, 100; KCl, 45; $MgCl_2$, 1; HEPES, 5; EGTA, 10; K-ATP, 3, or alternatively, KCl, 110; KOH, 40; HEPES, 10; EGTA, 10; TEACl, 20; K-ATP, 3, when measuring potassium or calcium currents, respectively (pH=7.2 in both cases). Membrane currents were 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. 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 using a 333-kHz A/D converter (Digidata-1200; Axon Instruments) under software control (pClamp 6.0; Axon Instruments). Data were stored on video tape for later analysis. The experimental protocol for each measurement is described where pertinent in the Results section.

Measurement of intracellular Ca^{2+} transients. $[Ca^{2+}]_i$ transients were recorded in voltage-clamped myocytes (Szigeti et al. 1998). Cells were loaded with the fluorescent indicator dye Fura-2 through the patch pipettes containing 100 μM K_5Fura-2 and illuminated alternatively at two wavelengths (340 nM and 380 nM). The excitation wavelengths were selected by the dual monochromator system of the Deltascan-1 apparatus (Photon Technology International). The emitted fluorescence was collected from a restricted area of the visual field, set by a variable slit. This area typically covered the total two-dimensional image of the cell and thus provided an optical signal that reflected the average $[Ca^{2+}]_i$. The intensity of the emitted fluorescence was measured by a photomultiplier and was not filtered. Background fluorescence was assessed in cell-attached mode prior to the establishment of the whole-cell configuration and was subtracted from the emitted fluorescence for both 340-nM and 380-nM excitations. Data acquisition was controlled by the OSCAR software (Photon Technology International) using a sampling frequency of 200 Hz. $[Ca^{2+}]_i$ is presented as a fluorescence ratio ($F_{340/380}$), corrected to the background, without calibration (Grynkiewicz et al. 1985).

Measurement of contractility. Contractility was measured in thin trabeculae dissected from the right ventricle and individually mounted in the experimental chamber. These preparations were superfused with Tyrode solution at a flow rate of 10 ml/min. One end of each preparation was fixed to the bottom of the chamber, its opposite end was attached to the lever arm of a mechanoelectronic transducer device. The preparations were paced at 1 Hz applying extracellular stimuli of twice the diastolic threshold in amplitude, which were mediated by a pair of platinum electrodes. Before the application of ET-1 for 30 min, each preparation was allowed to equilibrate for 90 min in Tyrode solution in order to achieve steady-state conditions.

Results are expressed as mean ± SEM values in the figures and the text. Student's *t*-test for paired and unpaired data was applied following ANOVA to determine statistical significance. Changes were considered significant when P was less than 0.05.

The entire investigation conforms 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 with the principles outlined in the Declaration of Helsinki.

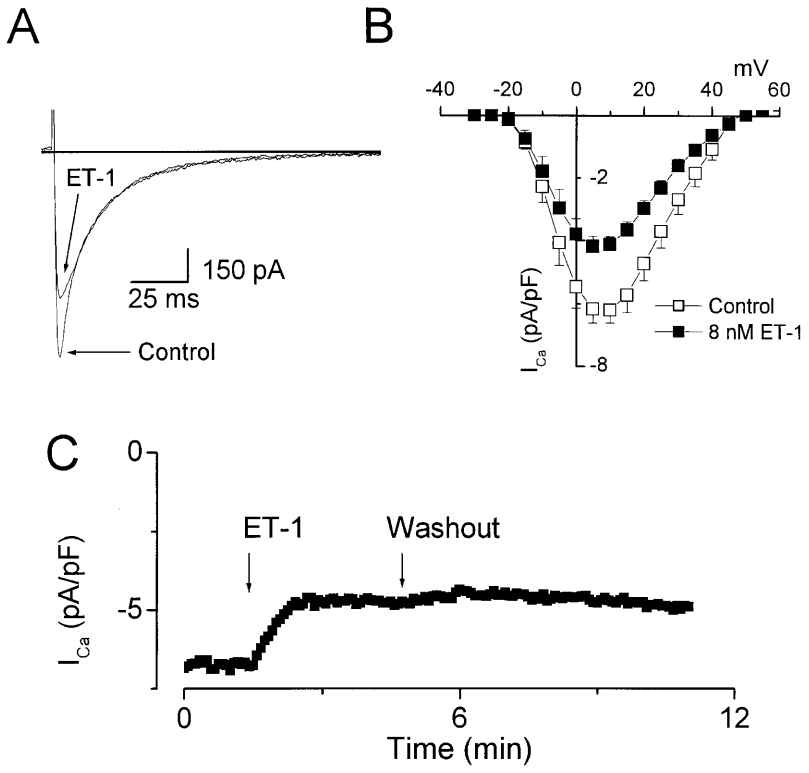

Fig. 1A–C Effect of ET-1 on calcium current in untreated myocytes. **A** Superimposed I_{Ca} records measured at +5 mV before and 5 min after superfusion with 8 nM ET-1. **B** Current-voltage relationship obtained for peak I_{Ca} in absence and presence of ET-1. The membrane was depolarized to potentials increasing from –35 mV up to +60 mV in 5-mV steps for 400 ms, the holding potential was –40 mV. *Symbols* and *bars* represent mean ± SEM values obtained in six cells. **C** A representative experiment showing the time course of development of ET-1 effect on peak I_{Ca} and lack of reversibility upon washout. Peak I_{Ca} was measured at +5 mV

Results

Effects of ET-1 in untreated preparations

I_{Ca} was measured at a rate of 0.2 Hz using depolarizing voltage pulses of 400 ms duration clamped from a holding potential of –40 mV to test potentials increasing from –35 mV up to +60 mV in 5-mV steps. Families of I_{Ca} were recorded before and 5 min after ET-1 treatment. Superfusion with 8 nM ET-1 decreased significantly I_{Ca} at all membrane potentials studied. At +5 mV, I_{Ca} was reduced by ET-1 from –6.2±0.45 pA/pF to –4.2±0.31 pA/pF (reduction of 32.3±4.8%, $P<0.05$, $n=6$). ET-1 caused no change in the time course of inactivation of I_{Ca} (Fig. 1A). Similarly, no change was observed in the voltage dependence of activation of I_{Ca}; current-voltage relationships obtained for peak I_{Ca} in the presence and absence of ET-1 were proportional (Fig. 1B). The effect of ET-1 on peak I_{Ca} developed rapidly (within 2 min) and was not reversible within a 7-min period of superfusion with ET-1-free Tyrode solution (Fig. 1C). No decay of I_{Ca} was observed throughout the experiments, indicating that lack of recovery upon washout may not be ascribed to run-down.

The steady-state current-voltage relationship of the membrane was studied between –130 mV and +60 mV at the end of 400-ms test pulses arising from a holding potential of –80 mV (Fig. 2A). At potentials more negative than –50 mV the current is most likely carried by I_{K1}. No significant change of this current was observed in the presence of 8 nM ET-1, since I_{K1} current densities were –15.9±1.4 pA/pF and –13.6±1.3 pA/pF at –130 mV before and after ET-1, respectively (N.S., $n=5$).

I_{to} was measured at a rate of 0.2 Hz using depolarizing voltage pulses of 400 ms duration clamped from a holding potential of –80 mV to test potentials ranging between –30 mV and +60 mV (Fig. 2B). Each test pulse was preceded by a short (5 ms) depolarization to –40 mV in order to inactivate sodium current. ET-1 failed to change the peak amplitude of I_{to} (14.3±0.14 pA/pF and 14.3±0.32 pA/pF at +50 mV before and after application of ET-1, respectively, N.S., $n=6$). Similarly, no change was caused by ET-1 in the time course of inactivation of I_{to}; inactivation time constants of 6.9±0.2 ms and 6.6±0.2 ms were obtained at +50 mV before and after ET-1, respectively (N.S., $n=7$). When studying steady-state inactivation of I_{to}, test pulses to +50 mV were preceded by a set of prepulses clamped to various voltages between –100 mV and +10 mV. Peak currents measured after these prepulses were normalized to the peak current measured after the –100-mV prepulse and plotted against the respective prepulse potential. As shown in Fig. 2C, steady-state inactivation curves, obtained in absence and presence of ET-1, were almost identical (midpoint potentials and slope factors were –25.9±1.4 mV vs. –26.7±0.4 mV, and 2.9±0.9 mV vs. 3.5±0.5 mV, respectively, N.S., $n=3$). Recovery kinetics of I_{to} was determined using paired-pulse protocol, each clamped to +50 mV (Fig. 2D). I_{to} was fully inactivated by the first pulse, and recovery of the current during repolarization to –80 mV was tested by the second pulse, while the interpulse interval was increasead up to 500 ms. The recovery time constant was obtained by monoexponential

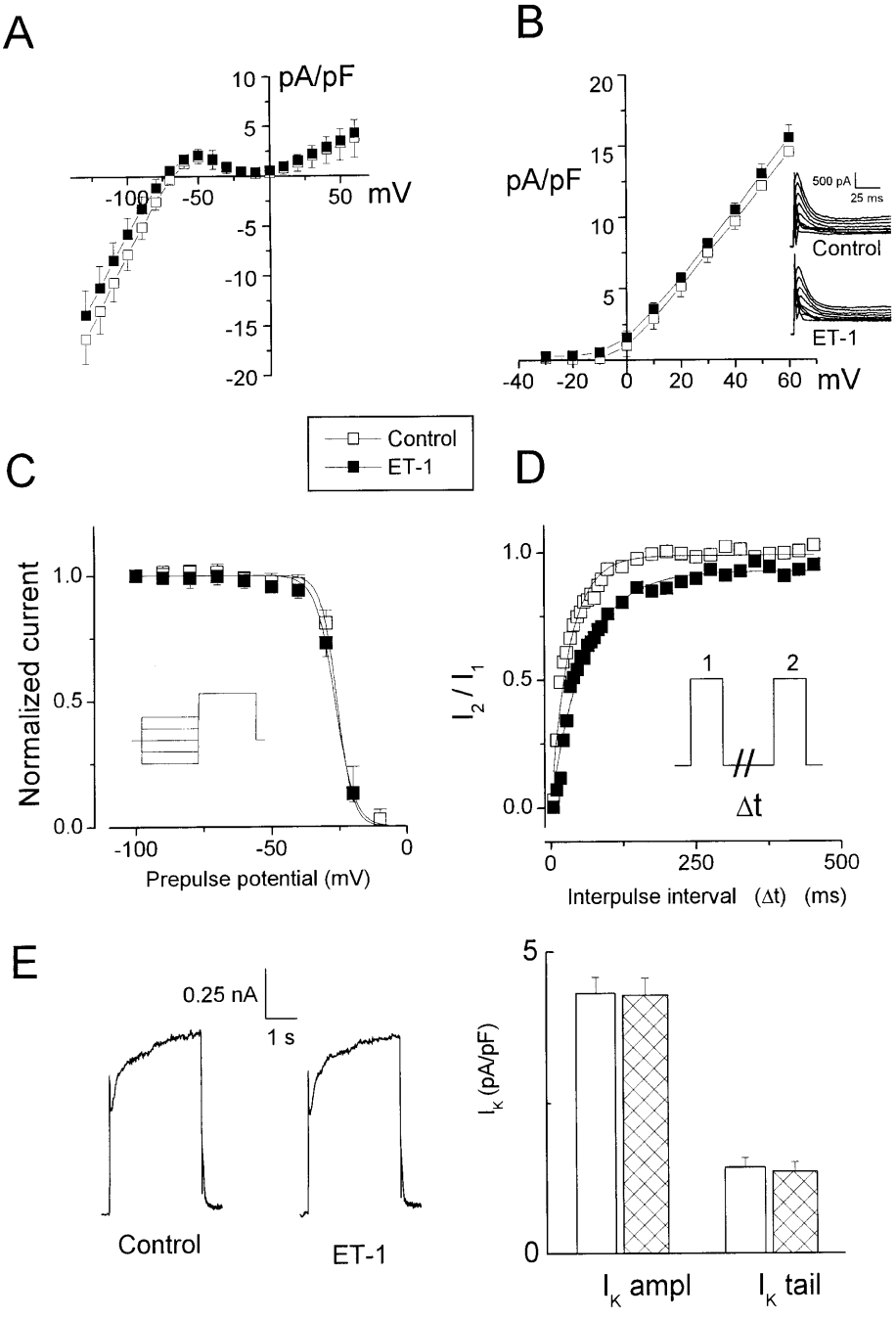

Fig. 2A–E Effect of ET-1 on potassium currents in untreated myocytes. *Open* and *filled symbols* represent control records and ones taken in the presence of 8 nM ET-1, respectively. I_{Ca} was blocked by 0.25 mM $CdCl_2$. **A** Steady-state current-voltage relationship of the cell membrane before and after ET-1 treatment (n=5). Currents were measured at the end of test pulses of 400 ms duration, clamped to voltages indicated in the *abscissa*. The current was carried by I_{K1} at potentials more negative than –50 mV. **B** Peak amplitude of I_{to} measured in six cells during depolarizations to potentials ranging between –30 mV and +60 mV and arising from the holding potential of –80 mV. The *inset* shows families of I_{to} obtained in absence and presence of ET-1. **C** Voltage dependence of inactivation of I_{to}. Test pulses to +50 mV were preceded by a set of prepulses clamped to various voltages between –100 mV and +10 mV. Peak currents measured after these prepulses were normalized to the peak current measured after the –100-mV prepulse and plotted against the respective prepulse potential (n=3). *Solid curves* were obtained by fitting data to the Boltzmann equation. **D** Recovery kinetics of I_{to} studied using paired-pulse protocol (*inset*) in three myocytes. I_{to} was fully inactivated by the first pulse, and recovery of the current during repolarization to –80 mV was tested by the second pulse. Both depolarizations were applied to +50 mV. The interpulse interval was varied between 10 ms and 500 ms. *Solid curves* show the results of monoexponential fitting of I_2/I_1 ratios plotted against their respective interpulse interval. **E** Original records of I_K (*left*) and amplitude of the fully activated current and the tail current (*right*) in absence (*open columns*) and presence of 8 nM ET-1 (*crossed columns*) studied in seven myocytes. I_K was activated during depolarizations to +60 mV for 3 s. The decaying current tails were recorded after repolarization to the resting potential of –40 mV

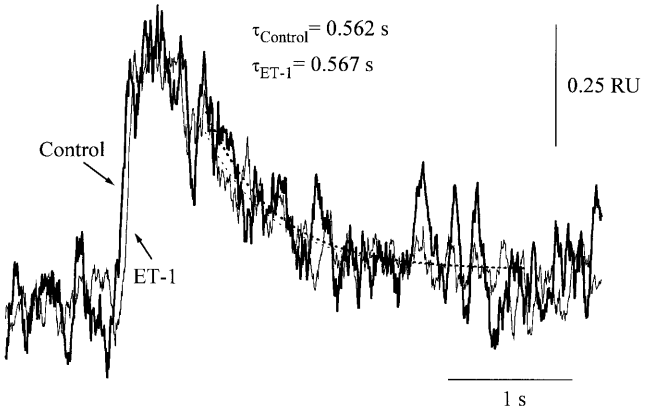

Fig. 3 Intracellular Ca^{2+} transients recorded in a voltage-clamped canine ventricular cell previously loaded with the fluorescent calcium indicator dye Fura-2. $[Ca^{2+}]_i$ was expressed as relative units (*RU*) of the fluorescent ratio, $F_{340/380}$. Depolarizations to 0 mV were applied for 350 ms at a frequency of 0.2 Hz before and after application of 8 nM ET-1. The descending phase of each $[Ca^{2+}]_i$ transient was fitted to an exponential function in order to estimate the time constant for $[Ca^{2+}]_i$ decay (*dashed lines*)

fitting of I_2/I_1 data plotted against the interpulse interval. ET-1 significantly increased the recovery time constant of I_{to} (from 26.5±4.6 ms to 59.5±1.8 ms, $P<0.05$, $n=3$).

I_K was activated using depolarizing voltage pulses of 3 s duration clamped from the holding potential of –40 mV to the test potential of +60 mV. The decaying tail current was recorded at –40 mV after the end of the test pulse (Fig. 2E). Although this protocol fails to differentiate precisely between the two components of I_K (i.e., I_{Kr} and I_{Ks}), the current activated during the 3-s depolarization represents I_{Ks} better than I_{Kr}, whereas the tail current is mainly determined by I_{Kr} in canine ventricular cells (Varró et al. 2000). In the seven myocytes studied, no change was caused by 8 nM ET-1 in the amplitude of the depolarization-activated current (4.31±0.27 pA/pF vs. 4.28±0.29 pA/pF), nor in tail current amplitude (1.43± 0.15 pA/pF vs. 1.36±0.16 pA/pF).

$[Ca^{2+}]_i$ transients were studied in voltage-clamped myocytes by applying depolarizations to 0 mV for 350 ms. Repetitive depolarizations to this potential with a frequency of 0.2 Hz resulted in a steady-state loading of the sarcoplasmic reticulum with calcium indicated by stable fluorescent intensity as a function of time. $[Ca^{2+}]_i$ was expressed as relative units of the fluorescent ratio, $F_{340/380}$. Neither systolic nor diastolic fluorescent ratio was altered significantly by 8 nM ET-1 (1.319±0.04 vs. 1.364± 0.06 and 1.007±0.004 vs. 1.009±0.06, respectively, both N.S., $n=5$). The descending phase of $[Ca^{2+}]_i$ transients was fitted to an exponential function in order to estimate the time constant for $[Ca^{2+}]_i$ decay (Fig. 3). ET-1 had no effect on the decay time constant of $[Ca^{2+}]_i$ (558±30 ms and 563±80 ms, before and after ET-1, respectively, N.S., $n=5$).

Effect of ET-1 on contractility was studied in canine ventricular trabeculae. Thirty-minute superfusion with 8 nM ET-1 failed to alter the contractile force (1.22±0.14 mN vs. 1.21±0.17 mN, N.S., $n=5$) in these preparations.

Effects of ET-1 in preparations with elevated intracellular cAMP level

Isoproterenol is known to increase both I_{Ca} and I_K in various mammalian cardiac preparations. In our experiments I_{Ca} was increased from –6.3±0.2 pA/pF to –16.6±2.3 pA/pF by 50 nM isoproterenol (263±29% of control, $P<0.05$, $n=5$). Application of 8 nmol/l ET-1 in the presence of isoproterenol reduced peak I_{Ca} from –16.6±2.3 pA/pF to –13.6±2.1 pA/pF ($P<0.05$); however, this reduction was only 17.8±2%, a value significantly less than obtained in untreated cells. Although the effect of isoproterenol on I_{Ca} was fully reversible, the effect of ET-1 was not eliminated after removal of the peptide (Fig. 4A).

Depolarization-activated I_K as well as I_K tails were increased by isoproterenol from 4.17±0.44 pA/pF to 8.87± 0.37 pA/pF and from 1.72±0.21 pA/pF to 3.29±0.18 pA/pF (213±18% and 191±16% of control, respectively, both $P<0.05$, $n=5$). ET-1 significantly decreased the amplitude of I_K activated during depolarization (from 8.87±0.37 pA/pF to 7.41±0.18 pA/pF) and I_K tails (from 3.29±0.18 pA/pF to 2.78±0.22 pA/pF) in the presence of isoproterenol (reduction of 16.2±1.65% and 15.6±4.8%, respectively, $P<0.05$, $n=5$). Again, the effect of ET-1 was practically irreversible, since removal of ET-1 caused no change in I_K in the continuous presence of isoproterenol (Fig. 4B). These results indicate that, in contrast to untreated preparations where ET-1 had no effect on I_K, the peptide was able to suppress the current enhanced previously by isoproterenol. In addition, ET-1 significantly reduced the effect of isoproterenol on I_K, when isoproterenol was applied following superfusion of ET-1 (Fig. 4C,D). In order to eliminate variance arising from individual differences in isoproterenol sensitivity of the myocytes, each cell was exposed to isoproterenol twice: first in the absence and then in the presence of ET-1. Pretreatment with 8 nM ET-1 decreased the isoproterenol-induced I_K amplitudes from 5.36±0.44 pA/pF to 3.65±0.28 pA/pF and I_K tails from 1.89±0.1 pA/pF to 1.21±0.1 pA/pF (reduction of 31.8± 4.8% and 36±3.4%, respectively, $P<0.05$, $n=3$). This reduction was twice in magnitude compared with the effect of ET-1 when isoproterenol was applied first. To exclude the possibility of time-dependent reduction of isoproterenol sensitivity, two further cells were exposed to isoproterenol twice both in the absence of ET-1 (not shown). Identical increases in I_K were observed during the first and second exposures separated by 30-min washout with isoproterenol-free Tyrode solution.

Maximal activation of PKA was achieved by application of 250 μM 8-bromo-cAMP in the pipette solution. In the presence of 8-bromo-cAMP ET-1 failed to alter significantly either I_{Ca} or I_K (Fig. 5A,B). I_{Ca} densities measured before and after 8 nM ET-1 were –23.9±0.6 pA/pF and –21.9±1.1 pA/pF (N.S., $n=6$). The corresponding values for I_K amplitudes and I_K tails were 9.99±0.19 pA/pF vs. 9.53±0.29 pA/pF (N.S., $n=6$) and 4.15±0.27 pA/pF vs. 3.96±0.24 pA/pF (N.S., $n=6$), respectively. These results indicate that cAMP-independent activation of PKA prevented the inhibitory action of ET-1 on both I_{Ca} and I_K.

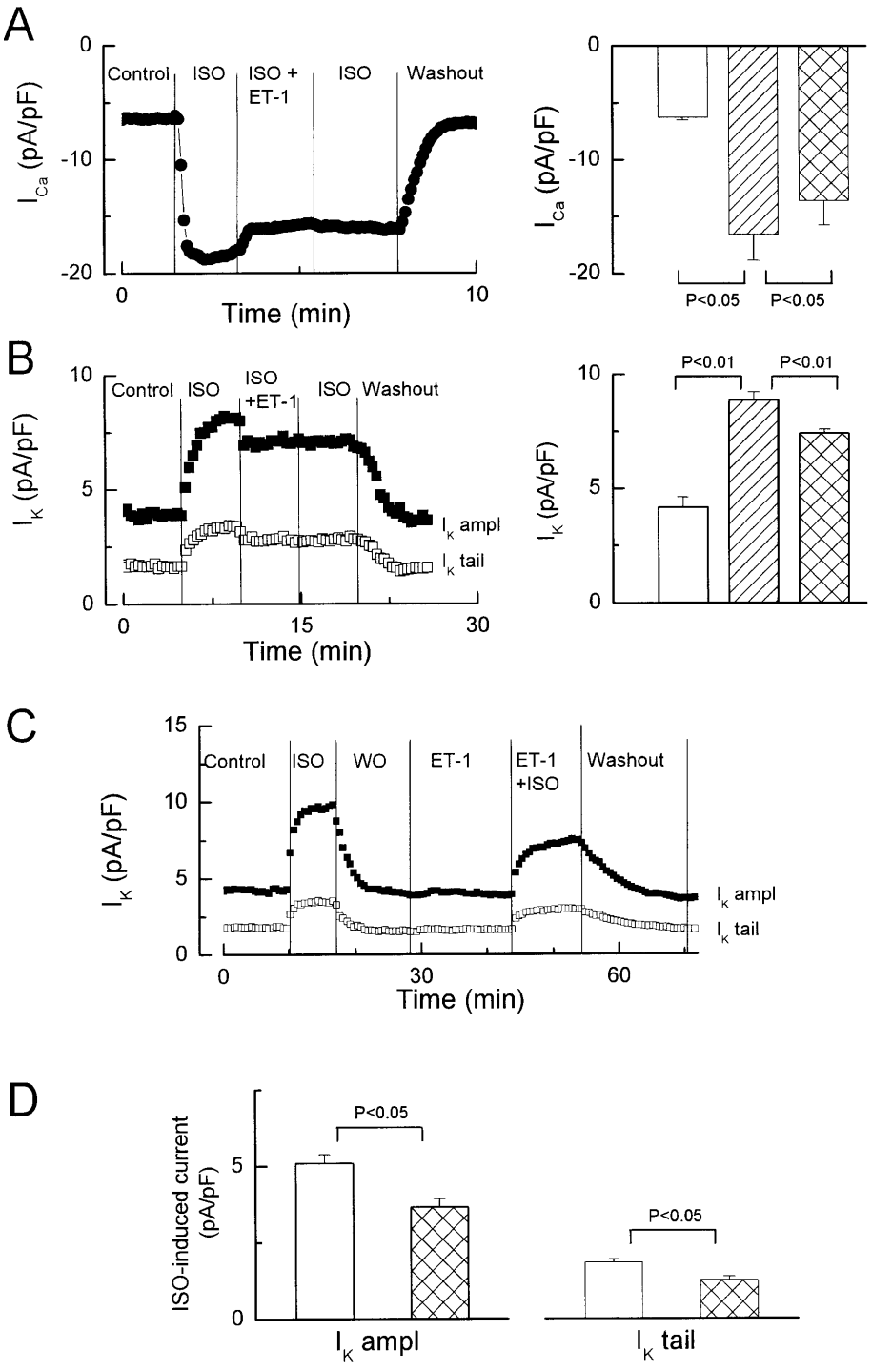

Fig. 4 Effect of ET-1 on **A** I_{Ca} and **B** I_K following pretreatment with isoproterenol. *Left panels* show representative experiment; average results, obtained in five cells in each series, are presented in *right panels*. *Open*, *striped* and *crossed columns* represent current amplitudes obtained in Tyrode solution, in the presence of 50 nM isoproterenol, and isoproterenol plus ET-1, respectively; washout data are not included. The fully activated current amplitudes (I_K *ampl*) and tail current amplitudes (I_K *tail*) are shown simultaneously in **B** *left panel*; **B** *right panel* shows only fully activated I_K amplitudes. **C,D** Effect of isoproterenol on I_K before and after ET-1 treatment in the same cell. *Open* and *crossed columns* in **D** represent isoproterenol-induced current amplitudes (both I_K *ampl* and I_K *tail* values) obtained in three cells before (*ISO alone*) and after ET-1 treatment (*ET-1 plus ISO*), respectively

Discussion

In this study we have shown that ET-1 is able to suppress both I_{Ca} and I_K in canine ventricular myocytes depending on the experimental conditions (i.e., resting cAMP levels, cAMP elevated by isoproterenol resulting in partial activation of PKA, and full activation of PKA by 8-bromo-cAMP). The most striking difference found in the action of ET-1 on I_{Ca} and I_K was that in untreated myocytes I_K was not affected but I_{Ca} was significantly depressed by ET-1. There are multiple mechanisms being already proposed to explain the effects of ET-1 in mammalian cardiac cells including inhibition of adenylate cyclase via PTX-sensitive inhibitory G protein by decreasing the activity of PKA (Hilal-Dandan et al. 1992; Vogelsang et al. 1994; Xie et al. 1996). Our results support this mechanism of action due to the loss of ET-1 effect in the presence of 8-bromo-cAMP.

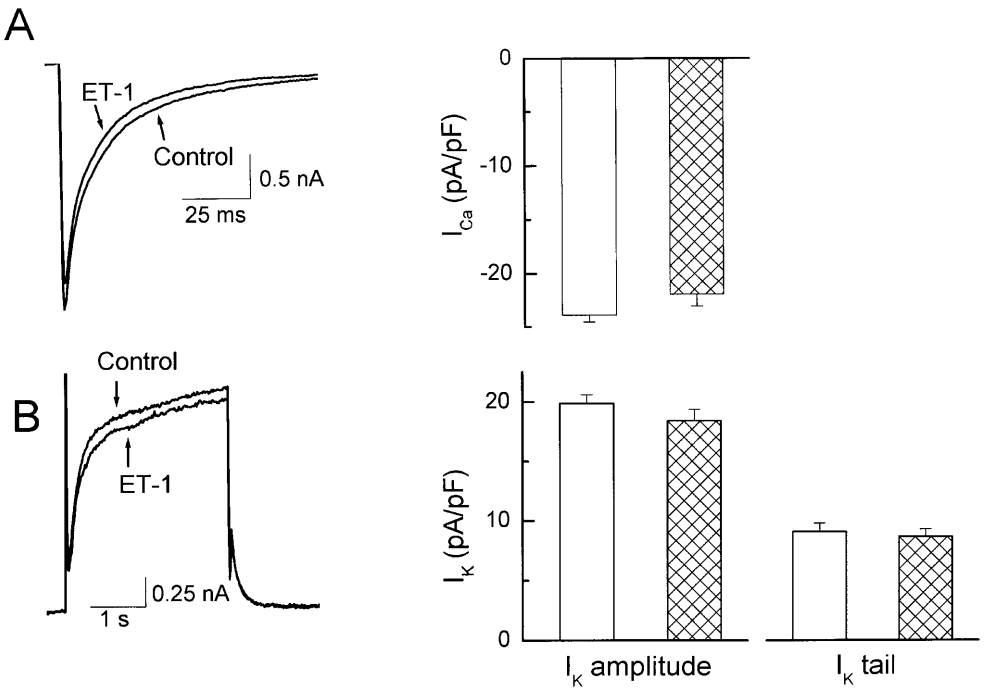

Fig. 5 Effect of 8 nM ET-1 on **A** I_{Ca} and **B** I_K in the presence of 250 μM 8-bromo-cAMP added to the pipette solution. Currents recorded before exposure to ET-1, but in the presence of 8-bromo-cAMP, are considered controls (*open columns*). *Crossed columns* denote data obtained 5 min after ET-1 treatment (n=6)

Differences in action of ET-1 on I_{Ca} and I_K can be well explained with the known differences in cAMP sensitivity of the two current systems (Nerbonne et al. 1984; Walsh et al. 1989; Szabo and Otero 1990), suggesting that the cAMP-dependent component of I_K is not activated at resting cAMP levels, but a significant fraction of cAMP-dependent I_{Ca} is active in unstimulated cells. Differences in phosphorylation were also demonstrated between K and Ca channels involving the contribution of more than one protein kinase (Hartzell et al. 1995). Recently subcellular compartmentalization of cAMP resulting in differences in cAMP-dependent modulation of Ca and K channels were reported (An et al. 1999). Any of these mechanisms may explain why I_{Ca} could be suppressed by ET-1 in untreated myocytes, while inhibition of I_K required previous application of isoproterenol. Whatever the reason is for these differences in cAMP sensitivity, the current-cAMP relationship seems to be shifted to the right in the case of I_K compared to I_{Ca}. Thus, in isoproterenol-treated myocytes I_K becomes sensitive to ET-1, suggesting that isoproterenol increased the cAMP level to the range which is sufficient to activate I_K, and ET-1 could decrease this elevated cAMP. In the case of I_{Ca} the ET-1-induced reduction of the current was relatively less in isoproterenol-treated (17.8%) than in untreated (32.3%) cells; however, this 17.8% reduction corresponded to 3 pA/pF in average, 1.5 times more than the 2 pA/pF value obtained in unstimulated myocytes. In the presence of 8-bromo-cAMP PKA is maximally activated, since phosphodiesterase cannot eliminate this cAMP derivative, resulting in a loss of sensitivity to endogenous cAMP, and consequently, the inhibitory action of ET-1 on both I_{Ca} and I_K.

In contrast to the marked ET-1-induced suppression of I_{Ca} observed in untreated myocytes, ET-1 left intracellular Ca^{2+} transients as well as force of contraction unaltered. The reason for this discrepancy in unclear at present; however, it may probably be related to other possible ET-1-initiated mechanisms compensating for the cAMP-dependent changes in Ca handling and contractility, such as the inhibition of phospholipase C, and consequently, decreasing intracellular DAG and IP_3 levels (Tohse et al. 1990). The ET-1-induced modification in the recovery kinetics of I_{to}, observed in the present study, might be consistent with reduction of phosphatidylinositol breakdown. Further studies are required to elucidate this point.

Comparing present results obtained in canine myocytes with those found in human cardiac cells, differences seems to be substantial. In the experiments of Cheng et al. (1995), performed in diseased human atrial and ventricular cells, the inhibitory action of ET-1 (10 nM) on I_{Ca} was more pronounced after isoproterenol treatment than in untreated myocytes. This is in contrast to our previous results obtained in undiseased human ventricular cells where the effect of ET-1 on I_{Ca} was identical with that reported in the present study for dog (Magyar et al. 2000). On the other hand, I_K was strongly suppressed by ET-1 in these untreated healthy human cells (inhibition of 80% was observed) in contrast to the lack of ET-1 effect on basal I_K in this study on dog. On the basis of these results canine cardiac myocardium may not be a suitable model for studying the actions of ET-1 in man.

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