Short Communication

Augmentation of the Delayed Rectifier Potassium Current by ET$_A$ Endothelin Receptor in Guinea Pig Atrial Myocytes

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Abstract.   The role of ET$_A$ endothelin receptor (ET$_A$R) in the regulation of the delayed rectifier potassium current (I_K) was examined in guinea pig atrial myocytes. Application of ET-1 (10 nM) together with an ET$_B$-receptor-selective antagonist, BQ-788 (300 nM), significantly increased the voltage-dependent activation of I_K without affecting its half-activation voltage or the slope factor, while it suppressed the calcium current (I_{CaL}) and displaced the time-independent background current to the outward direction. The data suggests that the augmentation of I_K contributes to the ET$_A$-receptor-mediated shortening of action potential duration, and hence to the negative inotropic response, in atria.

Keywords: ET$_A$ endothelin receptor, delayed rectifier K$^+$ current, atrium

We have previously reported that stimulation of the ET$_A$ endothelin receptor (ET$_A$R) causes a dramatic shortening of the action potential (AP) duration in atrial myocytes, for which the decrease in the calcium current (I_{CaL}) and the increase in the muscarinic potassium current ($I_{K(ACh)}$), through mediation by a pertussis toxin-sensitive GTP binding protein, are responsible (1, 2). The delayed rectifier potassium current (I_K) is also heavily involved in regulating the AP configuration, and hence in the electrical excitability and contractility, in the heart. However, the response in atrial I_K to selective ET$_A$R stimulation has not been described. In the present study, the role of ET$_A$R in I_K modulation was examined in guinea pig atrial myocytes to further elucidate the ionic mechanism for the ET$_A$R-mediated shortening of AP duration in atria.

All animal manipulations were carried out in accordance with the ethical guidelines for experimental animals of the National Institute of Health Sciences, Japan. Atrial myocytes of guinea pig heart were isolated according to our previously descrigbed method (1). Briefly, the whole heart was attached to a Langendorff apparatus and retrogradely perfused under a hydrostatic pressure of 80 cm H$_2$O with the following solutions in

sequence: 1) normal Tyrode's solution for 10 min, 2) 0-Ca^{2+} Tyrode's solution, in which CaCl$_2$ was omitted, for 15 – 20 min, and 3) 0-Ca^{2+} Tyrode's solution containing collagenase (42 U ml^{-1}; Yakult, Tokyo) for 15 – 20 min. All the solutions were kept at 37°C and continuously bubbled with 100% O$_2$. After this treatment, left and right atria were dissected out and transferred into ice-cold KB medium and then gently shaken with a large bore Pasteur pipette to dissociate atrial myocytes.

Membrane currents were recorded from single atrial myocytes by using the single electrode patch-clamp technique in the whole-cell configuration (1, 3), using a patch-clamp amplifier (EPC-7; List-Electronic, Darmstadt, Germany). All recordings were done at room temperature (25 – 27°C). Electrical signals were digitized at a sampling interval of 2400 μs after Bessel-filtering at 3 kHz and acquired in a PC/AT compatible computer (DESKPRO 386s; Compaq/Hewlett-Packard, Palo Alto, CA, USA) using CLAMPEX software (pCLAMP, version 5.5.1; Axon Instruments, Inc., Foster City, CA, USA) for later analyses by CLAMPFIT software (Axon Instruments, Inc.).

Normal Tyrode's solution contained 135 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 5 mM HEPES, 1.8 mM CaCl$_2$, 0.5 mM MgCl$_2$, 0.33 mM NaH$_2$PO$_4$, pH adjusted to 7.4 with NaOH. KB solution contained 50 mM L-glutamic acid mono-K salt, 10 mM taurine, 25 mM

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KCl, 10 mM KH_2PO_4, 0.5 mM EGTA, 10 mM HEPES, 3 mM $MgCl_2$, 10 mM glucose, pH adjusted to 7.4 with KOH. These solutions were bubbled with 100% O_2. The pipette solution contained 120 mM L-aspartic acid mono-K salt, 30 mM KCl, 5 mM EGTA, 5 mM HEPES, 4 mM ATP·2Na, 1 mM $MgCl_2$, pH adjusted to 7.2 with KOH. All these compounds were of analytical grade.

ET-1 was purchased from Peptide Institute, Inc. (Minoh), and BQ-788 (N-[N-[N-[(2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl]-1-(methoxycarbonyl)-D-tryptophyl]-D-norleucine monosodium) was from American Peptide Co. (Sunnyvale, CA, USA). After obtaining the control current-voltage (I-V) relation of the I_K activation, Tyrode's solution containing both ET-1 and BQ-788 was perfused, during which period I_{CaL} was monitored in order to determine the achievement of the maximum effect. Right after I_{CaL} had decreased to reach a steady level, the recording of the I-V

relation of I_K was started. All values are expressed as the mean ± S.E.M. Statistical difference between two groups was tested by ANOVA and Student's paired t-test, using the Statview software (Abacus Concepts, Inc., Berkeley, CA, USA). A value of $P<0.05$ was considered to be statistically significant.

ET_AR-selective stimulation, through using ET-1 at a relatively high but physiologically relevant concentration (10 nM) and an ET_B receptor-selective antagonist BQ-788 (300 M) in combination (4), greatly increased the maximum activation of I_K (Fig. 1). The ET_AR stimulation also caused a great outward shift in the holding current (Fig. 1a), being consistent with our previous finding of stimulation of $I_{K(ACh)}$ (1, 3). The voltage-dependence of the activation of I_K was examined by the isochronal current activation by measuring the tail current at the holding potential of −40 mV (Fig. 1b). It was found that ET-1 greatly increased the maximum
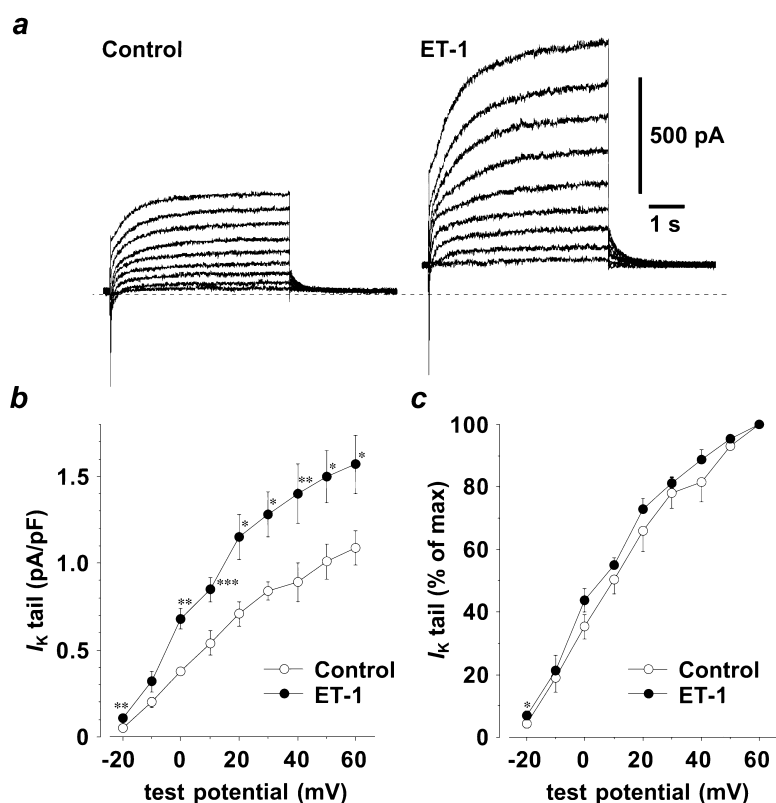

Fig. 1. Effect of ET-1, in the presence of BQ-788, on I_K of guinea pig atrial myocytes. a: Typical superimposed recordings of I_K. Current traces obtained during the I-V relation studies, each in the absence (Control) and presence of ET-1 (10 nM) plus BQ-788 (300 nM) are illustrated. Dotted line indicates the zero current level. Similar observations were obtained in three other cells. b and c: Summary of the inhibitory effect of ET-1 on the voltage-dependent activation of I_K, as measured by its tail current at the holding potential of −40 mV. b: I-V relation of the density of I_K tail (pA/pF) before (Control) and after application of ET-1 plus BQ-788. c: Fractional activation of the I_K tail. The current density was normalized by regarding each of the maximum current sizes in the control and in the presence of ET-1 plus BQ-788 in each cell as 100%. To obtain the I-V relation of the activation of I_K, depolarizing test pulses of −20 to +60 mV of 5-s duration were applied from the holding potential of −40 mV, at an interval of 10 s with a 10-mV increment. Vertical bars in graphs indicate ± S.E.M. The P values for statistical significance are indicated by *, **, and *** for $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

I_K tail by $145.1 \pm 8.6\%$ (n = 4), which was comparable in magnitude to the first report for ET-induced augmentation of I_K in guinea pig ventricular myocytes (5), but apparently greater than that reported later (6) where the concentration of ET-1 was five-fold higher than that in the present study.

The kinetic parameters of the voltage-dependent activation of I_K was obtained by fitting the fractional activation of I_K with the Boltzmann's distribution function:

$$n_\infty = [1 + \exp\{(V_m - V_h) / K\}]^{-1}, (K<0)$$

where V_m is the membrane potential, V_h is the half-activation voltage corresponding to $n_\infty = 0.5$, and K is the slope factor (3, 7). It was found that ET-1 did not affect either V_h (11.2 ± 3.0 mV in the control, 6.8 ± 2.1 mV in ET-1, $P = 0.25$, n = 4) or K (-14.3 ± 1.1 mV in the control, -13.8 ± 0.4 mV in ET-1, $P = 0.63$, n = 4) despite the great increase in the maximum current activation (Fig. 1c). I_K in the heart consists of at least two components: I_{Kr} and I_{Ks}. In order to estimate which component of I_K was mainly affected by ET-1, I analyzed the voltage-dependence of the ET-1-induced current component of I_K. The 'difference current' was obtained by subtracting the control I_K recording from the I_K recording in the presence of ET-1 and BQ-788, the tail of which showed a V_h of 13.4 ± 4.2 mV and a K of -18.5 ± 1.1 mV (n = 4). These values were quite similar to those of I_{Ks}, but not I_{Kr}, in guinea pig atrial

myocytes (8), indicating that ET$_A$R stimulation most likely augmented, at least in a major part, I_{Ks} in guinea pig atrial myocytes. A pharmacological dissection of the current components with their specific inhibitors (8, 9) would facilitate obtaining conclusive information about the target, which awaits further study.

I_{Ks} is known to be regulated by two major intracellular signaling pathways: the cAMP- and protein kinase A (PKA)-dependent one and the calcium and protein kinase C (PKC)-dependent one (5, 7, 10, 11). In the heart, ET$_A$R both mediates reduction in intracellular cAMP through a pertussis toxin-sensitive GTP binding protein (1, 2, 12, 13) and stimulates the calcium-PKC pathway (12). Accordingly, stimulation of ET$_A$R has been reported to modulate I_K in a biphasic manner with an initial decrease and a later increase, mediated, respectively, through reduction in intracellular cAMP and by the calcium-PKC pathway in guinea pig ventricular myocytes (6). The inhibition of I_K was not observed in the present study. One possible explanation may come from the difference in the temperature sensitivity of the two pathways; the cAMP-PKA pathway is highly temperature-dependent, therefore possibly being masked at room temperature, whereas the calcium-PKC pathway is not (10).

The consequences of the modulation of I_{Ks} by these two ET$_A$R-mediated intracellular pathways are distinguishable by the changes in the voltage-dependence of
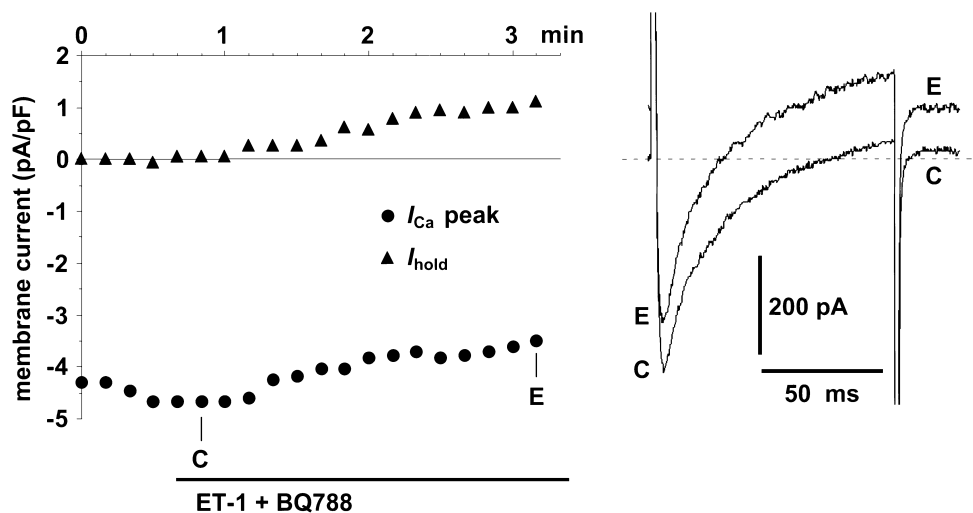

Fig. 2. Inhibition of I_{CaL} by mixed application of ET-1 and BQ-788. I_{CaL} was induced repetitively, with the interval of 10 s, by depolarizing command pulses of 100-ms duration at +10 mV from the holding potential of −40 mV. The horizontal bar indicates the combined application of ET-1 (10 nM) and BQ-788 (300 nM). On the right are superimposed current traces of I_{CaL}, obtained at the time marked by "C" (Control) and "E" (ET-1 plus BQ-788) on the left panel. This particular recording was obtained from the same cell as that illustrated in Fig. 1a, between the "Control" and "ET-1" recordings of the voltage-dependent activation of I_K. Similar recordings were obtained from a total of four different cells, where the peak I_{CaL} was significantly decreased from -3.7 ± 0.7 to -2.6 ± 0.5 pA/pF ($P<0.05$, n = 4) and the holding current was significantly increased from 0.5 ± 0.3 to 1.3 ± 0.3 pA/pF ($P<0.05$, n = 4).

the current activation; the cAMP-PKA pathway causes a leftward shift in V_h (7), whereas the calcium-PKC pathway does not (10, 11). The ET_AR stimulation increased I_{Ks} without accompanying a shift of V_h in the present study, which indicates that the calcium-PKC pathway is responsible for the response (5, 6, 10, 11).

The combined application of ET-1 plus BQ-788 significantly decreased I_{CaL} and shifted the holding current to the outward direction (Fig. 2), being quite consistent with our previous findings, which confirmed that the effect was indeed mediated by ET_AR (1, 3, 4). The result also supports that the increase in I_{Ks} is not secondary to an increase in I_{CaL}. The concentration of intracellular Ca^{2+} was kept low with 5 mM EGTA in the pipette in the present study, which may suggest involvement of the calcium-insensitive type of PKC (14) as reported for the modulation of the ATP-sensitive potassium channel (15). However, the present data does not exclude a possible involvement of the calcium-sensitive isoform of PKC, since stronger buffering of intracellular Ca^{2+} with 40 mM EGTA has been reported to abolish the ET-1-induced augmentation of I_K in guinea pig ventricular myocytes (5). Identification of the responsible PKC isoform awaits further study. The present result indicates that ET_AR stimulation increases I_{Ks} to contribute to the shortening of AP duration, which in turn may contribute to the negative inotropic response in guinea pig atria.

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