Br. J. Pharmacol. (1990), 99, 437–438 **SHORT COMMUNICATION**

Inability of endothelin to increase Ca^{2+} current in guinea-pig heart cells

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Effects of endothelin, a novel vasoconstrictor peptide derived from vascular endothelial cells, on cardiac contractility and membrane currents, were examined in guinea-pig cardiac preparations. Endothelin (3–1000 nM) produced a positive inotropic effect in papillary muscles in a concentration-dependent manner. In whole-cell voltage clamp recording, endothelin (250 nM) decreased the amplitude of Ca^{2+} current (I_{Ca}, 25.0 ± 6.6%) in ventricular myocytes. The endothelin-induced decrease in I_{Ca} was abolished by pretreatment with ryanodine (1 μM). These results suggest that endothelin does not activate cardiac sarcolemmal Ca^{2+} channels. The enhancement of the sarcoplasmic reticulum function may play an important role in the positive inotropic effect of endothelin.

Introduction Recently, a novel potent vasoconstrictor peptide derived from vascular endothelial cells, was discovered and termed endothelin (Yanagisawa *et al.*, 1988). In addition to its potent vasoconstrictor effect, Ishikawa *et al.* (1988) have shown that endothelin produces a positive inotropic effect in guinea-pig atria. They have also shown that the positive inotropic effect of endothelin was attenuated by a dihydropyridine Ca^{2+} antagonist, suggesting that the positive inotropic effect of endothelin may be mediated by an increase in transsarcolemmal Ca^{2+} influx. Therefore, we have evaluated directly the effects of endothelin on the Ca^{2+} current (I_{Ca}) of guinea-pig heart cells using the whole-cell voltage clamp technique.

Methods To measure cardiac contractile tension, the right ventricular papillary muscles were carefully dissected from guinea-pig heart according to the methods described by Hattori *et al.* (1986). The muscles were mounted vertically in water-jacketed baths containing Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at 30°C. The composition of Krebs-Henseleit solution (pH 7.4) was (in mM): NaCl 119, $CaCl_2$ 2.5, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9 and glucose 10. Isometric tension of the muscles was measured with a force transducer and recorded on a pen recorder. The muscles were paced electrically at a frequency of 0.5 Hz. The resting tension applied to the preparations was adjusted to 0.5 g. The concentration-response curves for the positive inotropic effect of endothelin were determined in a cumulative manner by increasing its concentration in steps of 0.5 log units. All experiments were carried out in the presence of propranolol (1 μM) to eliminate a possible modulation of the response by endogenously released noradrenaline.

To measure membrane currents, guinea-pig single ventricular cells were enzymatically isolated by the method described by Taniguchi *et al.* (1981). Membrane currents were recorded in the whole-cell voltage clamp mode of the patch-clamp technique (Hamill *et al.*, 1981). The pipette solution contained (in mM): KOH 110, KCl 20, $MgCl_2$ 1, K_2-ATP 5, K_2-creatine phosphate 5, aspartic acid 90–100 and EGTA 10. The pH was adjusted to 7.4 with 5 mM HEPES. For single cell experiments, the composition of the external solution was (in mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES-NaOH buffer 5.0 and the pH was adjusted to 7.4. The temperature of the perfusate was maintained at 34–36°C.

Endothelin was kindly given by Prof. T. Masaki, and also obtained from Peptide Institute Inc. (Osaka, Japan). Endothelin was dissolved in 10 mM Na-phosphate buffer (pH 7.0) to produce a 0.1 mM stock solution. Ryanodine (Progressive Agri Systems Inc., Wind Gap, PA, U.S.A.) was dissolved in dimethyl sulphoxide to give a 10 mM stock solution.

Statistical analyses of endothelin-induced changes were performed by use of Student's *t* test. Significance was established when the probability value was less than 0.05. All data are presented as means ± standard errors (s.e.).

Results Endothelin produced a positive inotropic effect in electrically paced guinea-pig papillary muscles. The threshold concentration for endothelin was 3 nM and the concentration to produce the maximal effect was 1 μM. Endothelin at a concentration of 1 μM increased the contractile tension by 219 ± 55% of the control values ($n = 6$, $P < 0.01$).

In order to examine the effects of endothelin on I_{Ca}, we applied the whole-cell configuration of the patch-clamp technique to single ventricular cells of guinea-pigs. The cells were held at −37 mV to avoid the influence of the fast Na^+ current. The I_{Ca} was elicited by a 300 ms depolarizing test pulse to +3 mV from the holding potential at 0.1 Hz. After the external application of 250 nM endothelin, I_{Ca} gradually decreased and reached a steady level within 3–5 min. In 8 cells, endothelin decreased the amplitude of I_{Ca} (which was defined as the difference between the peak current and the current at the end of test pulse) by 25.0 ± 6.6% of the control values ($P < 0.01$). Actual current traces and time course of the decrease in I_{Ca} of a representative experiment are shown in Figure 1b(i), (ii) and Figure 1a. The current-voltage relation of I_{Ca} obtained in the presence of endothelin showed that I_{Ca} decreased at every membrane potential tested from −27 mV to +63 mV (data not shown).

The endothelin-induced decrease in I_{Ca} was abolished by pretreatment with ryanodine, a specific inhibitor of sarcoplasmic reticular (SR) function (Sutko & Kenyon, 1983). In the presence of 1 μM ryanodine, I_{Ca} remained unchanged until 5 min after the application of 250 nM endothelin, as shown in Figure 1a and Figure 1b(iii and iv). In 6 cells pretreated with 1 μM ryanodine, 250 nM endothelin decreased the amplitude of I_{Ca} by only 2.9 ± 10.3% of control values (NS).

Discussion As previously demonstrated in guinea-pig left atria (Ishikawa *et al.*, 1988), endothelin increased contractile tension in guinea-pig papillary muscles in a concentration-dependent manner. However, we found that endothelin decreased I_{Ca} rather than increased it in guinea-pig single ventricular cells at a concentration of 250 nM at which the inotropic response to endothelin was submaximal in the guinea-pig

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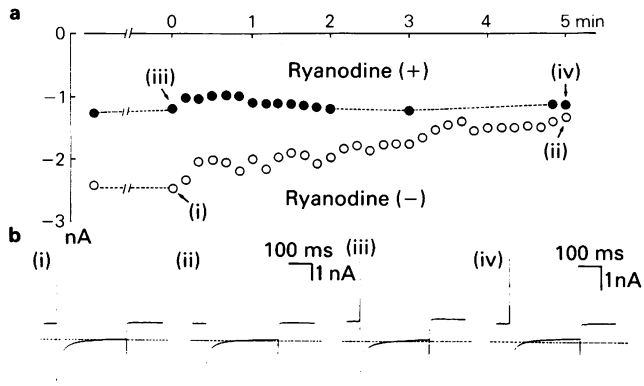

Figure 1 Effect of endothelin on I_{Ca} in the absence and presence of 1 μM ryanodine. (a) Time course change of an amplitude of I_{Ca} after 250 nM endothelin, which was given at time 0. The amplitude of I_{Ca} is defined as the difference between peak and steady-state current obtained by a 300 ms depolarizing test pulse to 3 mV from the holding potential of −37 mV. The test pulse was applied at 0.1 Hz. (○) No ryanodine present; (●) in presence of ryanodine 1 μM. (b) Representative examples of the actual current records at the time indicated in (a) (i–iv) are shown. The broken lines indicate zero current level.

papillary muscles. It is unlikely that the reduction in I_{Ca} produced by endothelin is due to its direct action on sarcolemmal Ca^{2+} channels because the peptide did not affect I_{Ca} in the presence of ryanodine.

It is not surprising to find that endothelin decreased rather than increased I_{Ca} in guinea-pig heart cells: if endothelin elevates intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in some way other than activating Ca^{2+} channels, the elevation of $[Ca^{2+}]_i$ may produce a decrease in I_{Ca} (Ca^{2+}-inactivation) (Lee *et al.*, 1985). In the present study, we showed that ryanodine abolished the endothelin-induced decrease in I_{Ca}. This result suggests that endothelin may influence SR-function. Therefore, it is possible that a source of intracellular Ca^{2+} increased by endothelin is SR but not Ca^{2+}-influx through sarcolemmal Ca^{2+} channels. As cell membranes are not readily permeable to polypeptide compounds, the action of endothelin on SR seems to require some intracellular second messengers.

Yanagisawa *et al.* (1988) have speculated that endothelin may be an intrinsic Ca^{2+}-agonist which directly activates the L-type-Ca channels, the speculation being based on the observation that the dihydropyridine Ca^{2+} antagonist nicardipine, at a relative high concentration, slightly but significantly shifted the concentration-response curve for the positive inotropic effect of endothelin in guinea-pig left atria (Ishikawa *et al.*, 1988). On the other hand, Gu *et al.* (1989) recently reported that endothelin failed to bind to any calcium antagonist binding sites associated with L-type calcium channels in rat cardiac membrane fragments. The present observation that endothelin produced no direct effect on I_{Ca} in the presence of ryanodine in guinea-pig heart cells clearly indicates that endothelin can increase the cardiac contractile tension without activating Ca^{2+} channels.

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