Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria

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Ishikawa, Tomohisa, Masashi Yanagisawa, Sadao Kimura, Katsutoshi Goto, and Tomoh Masaki. Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria. Am. J. Physiol. 255 (Heart Circ. Physiol. 24): H970–H973, 1988.—Endothelin (ET), a novel 21-amino acid peptide isolated from the culture supernatant of porcine aortic endothelial cells, has been shown to be the most potent of all known vasoconstrictor substances. The purpose of the present study was to investigate the effects and the mode of actions of ET on the heart. ET exerted a positive inotropic effect in a dose-dependent manner on the electrically driven left atria of guinea pigs. The ET-induced response was of slow onset and characteristically long lasting. The half-maximal effective dose of the ET-induced response was about 1 nM, indicating that ET is one of the most potent cardiotonic substances. The response was presumably caused through direct actions of ET on the myocytes, since adrenergic, histaminergic, and serotoninergic antagonists showed no effect on the response. The dose-response relationship of ET for the positive inotropic effect was displaced to the right in a parallel fashion by 0.3 μM nicardipine. In the left atria depolarized with 22 mM KCl, ET exhibited a positive inotropic effect with the slow response action potentials in response to the electrical pacing. These results suggest that the ET-induced response on the left atria is intimately related to the influx of extracellular Ca²⁺.

METHODS

Male albino guinea pigs (300–400 g) were anesthetized with pentobarbital sodium (50 mg/kg ip), and the hearts were quickly removed and immersed in an ice-cold Krebs-Ringer solution of the following composition (in mM): 113 NaCl, 4.8 KCl, 2.2 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHC₀₃, and 5.5 glucose. After adhering tissues were removed, left atria were dissected from the hearts.

Contraction experiments. Preparations were suspended by cotton threads in organ baths containing Krebs-Ringer solution (10 ml) maintained at 37°C and aerated with a mixture of 95% O₂-5% CO₂. One end was tied to a rod and the other to a force-displacement transducer (Nihon Kohden TB-612T, Tokyo, Japan) connected to an amplifier (Nihon Kohden AP-601G). The resting tension applied was 1 g (nearly maximal length). The isometric contraction was recorded on a thermal-pen recorder (Nihon Kohden WT687G). In some cases, the contraction was displayed on an oscilloscope (Nihon Kohden VC-9). Preparations were placed between a pair of platinum electrodes and electrically driven at 3.3 Hz with square wave pulses of 1 ms in duration and of an intensity sufficient to elicit contractions (usually 1 V) generated through a Nihon Kohden SEN-7103 stimulator. The electrical stimulation with these parameters did not bring about excitation of intramural nerves (5). All preparations were allowed to equilibrate for at least 2 h during which the bathing solution was replaced at 15-min intervals.

Electrophysiological experiments. The isolated left atrium was pinned with the endocardial surface uppermost in a tissue bath (30 ml). The Krebs-Ringer solution, aerated with a mixture of 95% O₂-5% CO₂, flowed...
through the chamber at a rate of 2-3 ml/min. The tissue bath temperature was maintained at 37°C. The preparation was stimulated electrically at 3.3 Hz with a pair of platinum electrodes by square-wave pulses of 1 ms in duration and a suprathereshold intensity of about 1.5 V. Glass capillary microelectrodes, filled with 2 M KCl and having resistances of 20–50 MΩ, were impaled in the cardiac cells. Potentials were amplified by a microelectrode amplifier (Nihon Kohden MEZ-8201) and displayed on an oscilloscope. The preparations were allowed to equilibrate for at least 3 h.

For the study of slow action potentials, the fast Na⁺ channels were inactivated by partial depolarization produced by perfusion with a 22 mM K⁺ Krebs-Ringer solution (isomolar substitution of K⁺ for Na⁺). The K⁺-depolarized atrium was stimulated electrically at 0.5 Hz with square-wave pulses of 1-ms duration and 10-V intensity.

Materials and statistics. ET was synthesized by Applied Biosystems model 430A peptide synthesizer and was identical to the natural peptide on the retention times of a reverse-phase high-performance liquid chromatography (HPLC) and an anion-exchange HPLC. ET was dissolved in a phosphate-buffered saline (pH 7.4) containing 0.05% bovine serum albumin. Appropriate vehicle controls showed no effect on the contractility of preparations. Drugs used were atenolol, bovine serum albumin (fraction V), cimetidine, isoproterenol hydrochloride, nicardpine (Sigma Chemical, St. Louis, MO); bunazosin (Eisai, Tokyo, Japan); diphenhydramine hydrochloride (Tokyo Kasei, Tokyo, Japan); ICI 118551 (Imperial Chemical Industries, Cheshire, UK); and methysergide hydrogen maleate (Sandoz, Basel, Switzerland). Values are expressed as means ± SE. Comparisons were made using the one-way analysis of variance (ANOVA) followed by the Bonferroni method (22), where comparisons with a common control were made. The level of statistically significant difference was P < 0.05.

RESULTS

Positive inotropic effects. As shown in Fig. 1, ET produced a positive inotropic response on the electrically driven left atria of guinea pigs in a dose-dependent manner. The maximal response to ET was 36% of that to isoproterenol and the half-maximal effective dose (ED₅₀) of ET was 1.08 nM (Table 1). The positive inotropic response to ET developed slowly (5–10 min to attain a steady state) and was almost irreversible even after repeated washings (120 min). The effects of ET were not affected by the blocker of biogenic amines (see legends of Fig. 1). Nicardpine (0.3 μM) significantly attenuated the inotropic effect of ET, where it caused a shift of the ET dose-response curve to the right.

As is seen in the oscilloscope records (Fig. 2), the positive inotropic action of ET was accompanied with little changes in the time course of tension development. Elevated external Ca²⁺ showed a similar effect with ET on twitch tension. In contrast, isoproterenol shortened the time to peak force and accelerated the rate of relaxation regardless of the magnitude of the induced positive

![Dose-response curves for positive inotropic effects of ET on guinea pig left atria in the absence (●; n = 8) or presence of...](http://ajpheart.physiology.org/)

**TABLE 1. ED₅₀ and maximum response to endothelin**

<table>
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<th>ED₅₀, M (95% CI)</th>
<th>Maximum,* %</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.08 x 10⁻⁹ (4.65 x 10⁻¹⁰ to 2.53 x 10⁻⁹)</td>
<td>35.6 ± 3.90</td>
</tr>
<tr>
<td>+ Blockers</td>
<td>9.53 x 10⁻⁹ (5.15 x 10⁻¹⁰ to 1.76 x 10⁻⁹)</td>
<td>42.4 ± 2.39</td>
</tr>
<tr>
<td>+ Nicardpine</td>
<td>2.33 x 10⁻⁴ (1.69 x 10⁻⁴ to 3.79 x 10⁻⁴)</td>
<td>33.2 ± 2.92</td>
</tr>
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ED₅₀, half-maximal effective dose with 95% confidence intervals given in parentheses. *% of the maximum responses to isoproterenol (10⁻⁶ M); values are means ± SE. tP < 0.05 from control (ANOVA following Bonferroni method).

Electrophysiological effects. Figure 3A shows traces of the action potentials recorded from both before and 2 min after application of ET (30 nM). The amplitude and duration of the plateau phase of the action potential were increased by ET.

In the atria, in which the fast Na⁺ channels were voltage inactivated by partial depolarization in elevated K⁺ (22 mM), the addition of ET (30 nM) either augmented or induced slow response action potentials (Fig. 3B).

DISCUSSION

The present study indicates that the endothelium derived peptide endothelin has not only a vasocontracting but also a positive inotropic effect. Initiation of the inotropic response to ET was in the range of low doses (~0.1 nM), and the maximal response was attained at around 30 nM. Therefore the molar potency of ET for the inotropic action seems to be the strongest of all the known cardiotonic agents, although the maximum response to ET was small compared with that of isoproteren-
enol. ET appeared to induce the inotropic response through its direct action on the myocytes, since the response was not affected by antagonists to norepinephrine (bunazosin, atenolol, and ICI 118,551), histamine (diphenhydramine and cimetidine), and serotonin (methysergide). The dose-response curve for ET was shifted to the right in an almost parallel manner by nicardipine, a dihydropyridine Ca\(^{2+}\) channel blocker, suggesting a competitive antagonism between ET and nicardipine.

The most prominent effect of ET on the electrophysiological properties of the atrial cells was an increase in the amplitude of the action potential plateau. It is thought that the plateau phase is produced by the slow inward current (\(I_{\text{slo}}\)) which is largely carried by Ca\(^{2+}\). To examine \(I_{\text{slo}}\) exclusively, the fast Na\(^{+}\) channel was inactivated by means of partial depolarization by elevating the external K\(^{+}\) concentration (14,19). Under this condition, ET augmented Ca\(^{2+}\)-dependent action potentials. It therefore seems likely that ET accelerated Ca\(^{2+}\) influx through the voltage-dependent Ca\(^{2+}\) channels, which are sensitive to dihydropyridine Ca\(^{2+}\) antagonists.

Increase in \(I_{\text{slo}}\) has been well known to be brought about by agents that produce an increase in the tissue content of adenosine 3',5'-cyclic monophosphate (cAMP) (20). However, the mechanical response to ET was not consistent with cAMP-mediated mechanism. Such substances as isoproterenol (16) and calcitonin gene-related peptide (8), which increase the cAMP level, induce a shortening of the time to peak force and an acceleration of the relaxation during each contraction via enhancing Ca\(^{2+}\) uptake into the sarcoplasmic reticulum (SR) (9). However, ET caused neither shortening of the time to peak nor acceleration of the relaxation, as in the case of the elevated external Ca\(^{2+}\). These results indicate that ET has no appreciable effect on Ca\(^{2+}\) uptake into SR, and therefore its effect is unlikely to be mediated by the cAMP system.

The inotropic response to ET was characterized by slow development and a long-lasting time course and was sensitive to dihydropyridine Ca\(^{2+}\) antagonists. Furthermore, the pattern of the increase in contractile response to ET was mimicked by the elevation of external Ca\(^{2+}\). Taken together, the effect of ET appears to be much similar to that of BAY K 8644. It is well known that BAY K 8644 increases Ca\(^{2+}\) influx by directly acting on the voltage-dependent Ca\(^{2+}\) channels, i.e., a Ca\(^{2+}\) channel activator (6,17). It has been reported that angiotensin II also exerts positive inotropic effects accompanying an increase in \(I_{\text{slo}}\). However, angiotensin II is distinguished from ET, since it produces a rapid increase in tension and is easily washed out (1,10).

At the discovery of BAY K 8644, Schramm and co-workers (17) proposed the possibility that there exist some endogenous factors which operate or modulate directly the voltage-dependent Ca\(^{2+}\) channel function in physiological or pathophysiological conditions. Consistent with this proposal, the results of the present study are compatible with our previous suggestion that ET may be one of the endogenous agonists of Ca\(^{2+}\) channels in the cardiovascular tissues (23).
REFERENCES


