Vol. 173, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1990 Pages 457-462

EVIDENCE FOR DIRECT ARRHYTHMOGENIC ACTION OF ENDOTHELIN

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Received October 16, 1990

SUMMARY: We studied electrophysiological effects of endothelin on canine cardiac tissues. Endothelin prolonged action potential duration and decreased spontaneous firing rate of the right bundle branch cells. At a concentration of $2x10^{-7}M$ the plateau phase of action potentials was flattened, followed by the abrupt occurrence of early afterdepolarizations (EADs). ET, at a concentration as low as $2x10^{-9}M$, was capable of inducing EADs although their incidence was low. The EADs were initiated from the membrane potential less negative than -30mV and were suppressed by nicardipine, suggesting the involvement of dihydropyridine-sensitive Ca^{2+} channels in the induction of EADs. Because EADs are considered to underlie certain types of arrhythmias endothelin per se may have arrhythmogenic action.

INTRODUCTION: Since the discovery of endothelin (ET)[1], a potent vasoconstrictor peptide produced by vascular endothelial cells, a variety of actions have been found for ET. These include actions on the heart, kidney and non-vascular smooth muscles[2]. ET has been shown in guinea pig atria to prolong action potential duration (APD) and to increase the contractile force[3]. In intact animals ET produces myocardial ischemia due to an intense coronary vasoconstriction and causes cardiac death[4,5]. Recently we showed that a close-coronary administration of ET elicited lethal arrhythmias in anesthetized rats[6]. Since these arrhythmias could not solely be attributed to myocardial ischemia, we speculated that ET per se had arrhythmogenic actions. In the present study we investigated electrophysiological effects of ET on canine cardiac tissues to clarify the mechanisms underlying arrhythmias caused by the agent.

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METHODS AND MATERIALS

Adult male mongrel dogs were anesthetized with sodium pentobarbital and were artificially ventilated. After thoracotomy the heart was rapidly removed and ventricular septa containing the right bundle branch were excised. The tissue preparation was mounted in an acrylic chamber filled with a 25ml Krebs-Henseleit solution of the following composition (mM): NaCl 126.7, $NaHCO_3$ 22.0, KCl 2.2, KH_2PO_4 1.8, $CaCl_2$ 2.5, $MgCl_2$ 0.5 and glucose 5.5. The solution was aerated with a mixture of 95%O_2 and 5%CO_2 gas and was maintained at 37°C. The transmembrane potentials were measured with a glass microelectrode, filled with 3M KCl and having resistance of 10-20MOhm. The preparations were exposed to $2x10^{-9}$, $2x10^{-8}$ or $2x10^{-7}$M of ET-1 or vehicle (0.05% bovine serum albumin, BSA), and action potentials were observed for 1hr.

Endothelin (ET-1, Peptide Institute, Osaka, Japan) was dissolved in 0.05% BSA. Bay k 8644 (Wako Pure Chemical, Osaka, Japan) and Nicardipine (Sigma) were dissolved in dimethyl sulfoxide and were diluted by distilled water.

RESULTS AND DISCUSSION

Action potentials recorded from the most upper portion of the right bundle branch showed a pacemaker activity. At lower concentrations of ET, $2x10^{-9}$ or $2x10^{-8}$M, APD gradually increased and reached a plateau 30-40min after exposure (Fig.1B.C). At a concentration of $2x10^{-7}$M of ET the plateau phase of action potential was flattened, followed by the abrupt occurrence of early afterdepolarizations (EADs) 24±6min after exposure to ET (N=5) (Fig.1D,E). EADs were elicited in 5 out of 6 preparations at this concentration and each action potential had a single EAD (in 4 out of 5 preparations) or multiple EADs (in 1 out of 5, see Fig.1E) which were not abolished by removing ET from the bathing solution.

EADs were initiated from a take-off potential of -24.5±2.6mV and reached a peak voltage of -8.9±2.2mV (N=5). The most negative take-off potential of EADs recorded was -34mV. Peak voltage of EADs never exceeded the zero potential. Action potential duration (APD) at 50% repolarization (APD_{50}) just prior to the induction of EADs was 460±26msec, which was about twice the control value.

ET at $2x10^{-9}$M, as well as at $2x10^{-8}$M, elicited EADs in 1 out of 6 preparations. The characteristics of EADs produced by lower concentrations of ET were similar to those by the highest one. The APDs of the two preparations in which EADs developed were much longer than the APDs of the others in which EADs did not developed, suggesting that the induction of EADs is associated with lengthening of APD, particularly flattering of plateau phase. Other parameters such as the maximum diastolic potential,

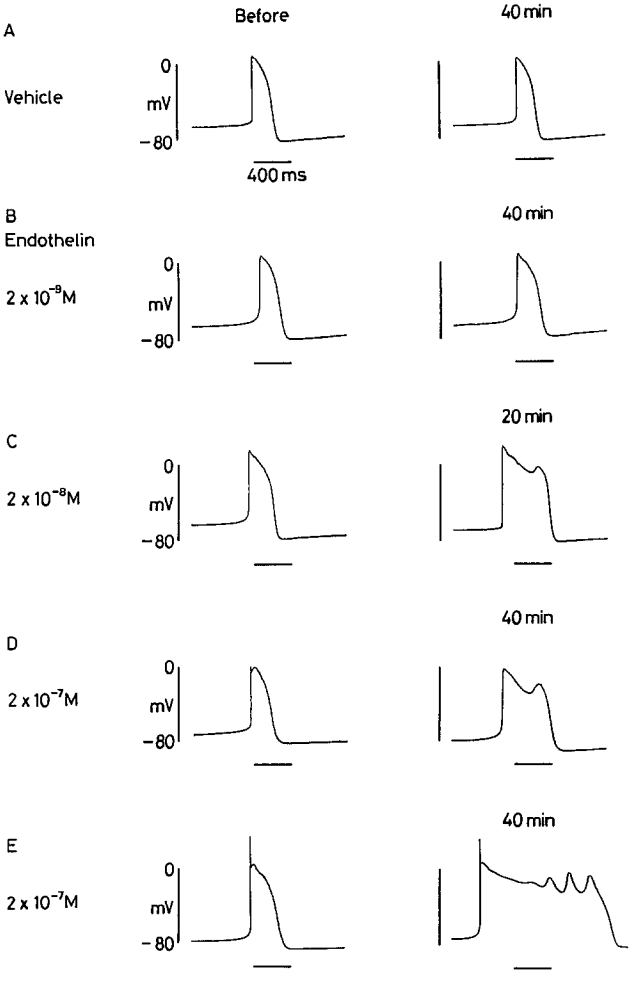

Fig. 1. Effects of endothelin and vehicle on action potentials recorded from the right bundle branch.

take-off potential and amplitude of action potentials were not affected by ET. Spontaneous firing rate was decreased by the highest concentration of ET, $2x10^{-7}M$.

It has been reported that ET indirectly activates the dihydropyridine-sensitive Ca^{2+} channels via the specific ET receptors, although the precise cellular mechanisms are not known[2,7]. We therefore examined the effects of nicardipine and Bay k 8644, a dihydropyridine Ca^{2+} channel antagonist and agonist, respectively, to examine if the activation of Ca^{2+} channels is involved in the induction of EADs by ET. In one study, EADs were initially generated by $2x10^{-7}M$ of ET, and $1x10^{-6}M$ of nicardipine was then added to the chamber. Nicardipine completely eliminated EADs, shortened the prolonged APD (Fig. 2, upper panel) and increased the firing rate slightly.

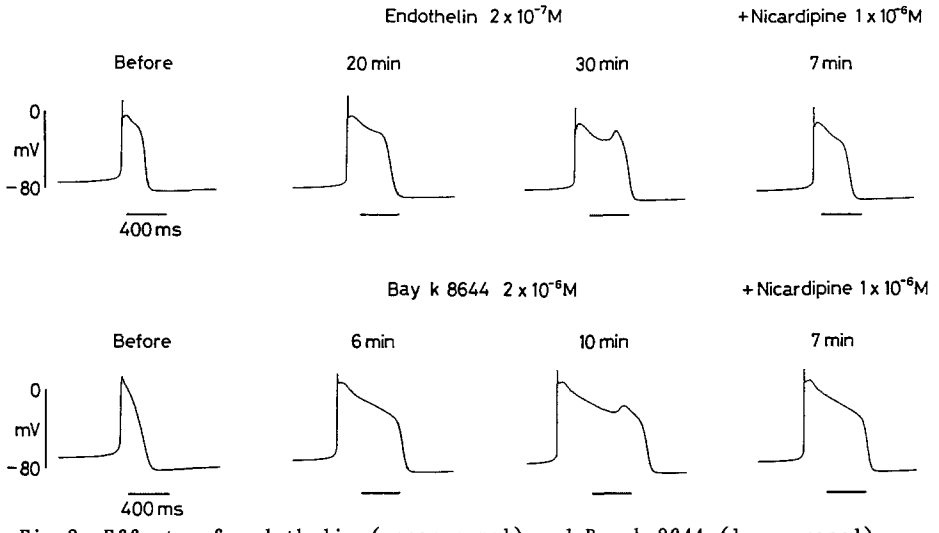

Fig. 2. Effects of endothelin (upper panel) and Bay k 8644 (lower panel) on action potentials recorded from the right bundle branch.

In other study, Bay k 8644 was added in a cumulative manner at concentrations of $2x10^{-8}$, $2x10^{-7}$ and $2x10^{-6}$M. The agent increased APD in a concentration related manner (Fig. 3) and elicited single or multiple EADs at the highest concentration in 1 out of 4 preparations. The EADs were suppressed and the prolonged APD was shortened by $1x10^{-6}$M of nicardipine (Fig. 2, lower panel). In other three preparations treated with Bay k 8644 EADs were not induced, although APD was markedly prolonged. Other parameters of action potentials were not affected by Bay k 8644 except firing rate which was increased slightly.

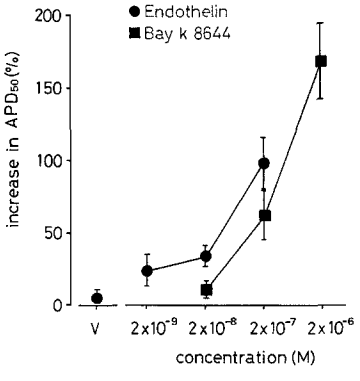

Fig. 3. Effects of endothelin (filled circle) and Bay k 8644 (filled square) on action potential duration (APD) recorded from the right bundle branch.
V: vehicle.
Abscissa: concentrations of endothelin and Bay k 8644.
Ordinate: percent changes in APD measured at 50% repolarization (APD_{50}). APD_{50} was measured before EADs were developed.

Recently, January et al. have shown in sheep purkinje fibers that Bay k 8644 induces EADs by increasing L-type Ca^{2+} "window" current during steady state[8,9]. EADs induced by ET and Bay k 8644 were similar in the following aspects:1)EADs occurred during the late portion of plateau phase. 2)EADs were always associated with prolongation of APD, which was long enough for the Ca^{2+} channels to recover from the inactive state. 3)Their take-off potential and peak potential lay near -30mV and 0mV, respectively, which roughly corresponded to the maximum activation and inactivation voltages of the Ca^{2+} channels[10]. In view of these similarities, it is conceivable that EADs elicited by ET involve the activation of L-type Ca^{2+} channels.

However, the electrophysiological actions of ET and Bay k 8644 were not entirely the same: 1)Bay k 8644 at $2x10^{-6}M$ produced a greater prolongation of APD than ET at $2x10^{-7}M$, whereas the incidence of EADs was much higher for ET (5/6 vs 1/4 for Bay k 8644)(Fig.3). 2)ET decreased the firing rate of the right bundle branch cells, whereas Bay k 8644 increased it. These observations suggest that ionic mechanisms other than Ca^{2+} channel activation may also be involved in the actions of ET.

Two subtypes of EADs have been suggested, based on the level of their take-off potentials[11]. The first type has a take-off potential less negative than -30mV and the second type more negative than -50mV. It is proposed that slow inward current is mainly involved in the first type of EADs and that Na^{+} "window" current and/or outward K^{+} current are involved in the second type. EADs induced by ET were of the first type. EADs are induced by a variety of substances such as Cs^{+} and quinidine that prolong APD in isolated cardiac tissues. They have been shown in intact animals to produce QT prolongation, polymorphous ventricular tachycardia and so-called "torsades de pointes"[12].

Several reports have shown that intracoronary administration of ET produces ventricular fibrillation (VF) in intact animals[4,5]. The authors of these reports have attributed VF to myocardial ischemia resulting from an intense coronary vasoconstriction. Recently, however, we demonstrated that close-coronary administration of ET elicited VF in anesthetized rats and that VF developed after myocardial ischemia subsided[6]. These observations

have lead us to speculate that ET per se has a direct arrhythmogenic action. The results of the present study suggested that this was the case.

Although the concentration for inducing EADs was high, ET may cause lethal arrhythmias in situations where a large amount of it is locally released in the coronary vascular bed. The arrhythmogenic action of ET together with potent coronary vasoconstrictor action implies that the peptide is involved in ischemic arrhythmias and cardiac sudden death.

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