The Arrhythmogenic Action of Endothelin in Rats

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Abstract—Endothelin (ET) was administered into the coronary ostia at doses of 0.1–1 μg/kg in anesthetized rats. The ST segment was depressed at doses below 0.5 μg/kg and was transiently elevated at 1 μg/kg. Ventricular arrhythmias developed at doses above 0.5 μg/kg. The arrhythmias that developed at 1 μg/kg were precipitated into ventricular fibrillation. At the time when the arrhythmias developed, the ischemic changes had already subsided. These results suggest that ET may have an arrhythmogenic action, which is not solely attributable to myocardial ischemia.

Endothelin (ET) is a newly discovered peptide that has a potent vasoconstrictor action (1). Because it is released in response to physiological stimuli such as thrombin, hypoxia and mechanical stretching, ET has been implicated in the pathogenesis of hypertension or coronary vasospasm, or both. In in vitro experiments, ET constricts the coronary artery (2), increases myocardial contractile force (3–5), and increases sino-atrial rate (6). It was recently demonstrated that ET, when injected directly into the coronary circulation, produced a coronary vasoconstriction and ST segment elevation in anesthetized dogs (7–10) and caused an aggravation of myocardial shortening and death resulting from cardiac insufficiency in anesthetized pigs (11). Thus, ET may be a potent cardiotoxic peptide in intact animals. The present study was designed to characterize the cardiotoxic actions of ET in anesthetized rats.

Male Sprague-Dawley rats weighing 300–360 g were anesthetized with inactin at 100 mg/kg, i.p. and intubated. The left femoral artery was cannulated for recording arterial blood pressure. The electrocardiogram (ECG) was picked up by the apex-base lead. In 3 out of a total of 11 rats, the left ventricular pressure (LVP), its first derivative (LVdp/dt) and left ventricular end-diastolic pressure (LVEDP) were measured through a 22 gauge needle inserted into the left ventricle. A stainless steel catheter (12) was introduced from the right carotid artery, and the tip of the catheter was fixed near the coronary ostia so that a bolus injection of methacholine, 1.2 μg/kg, produced the greatest ST segment elevation (+0.46±0.04 mV from −0.22±0.03 mV) in the ECG. Because the ST segment elevation elicited by methacholine has been shown to be due to myocardial ischemia by coronary vasospasm (12), a portion of the drug injected through this catheter was considered to enter the coronary circulation. We therefore designate this administration close-coronary administration in the present study.

In another series of experiments, ET was administered through the same cannula that was placed in the aorta distal to the coronary ostia in 3 rats (aortic administration). ET (ET-1, Peptide Institute, Osaka, Japan) was dissolved in saline containing 0.5% bovine serum albumin. Methacholine chloride (Sigma) was dissolved in saline. Close-coronary or aortic administration of the vehicle for ET did not produce any changes in systemic hemodynamics and ECG. All experimental data were expressed as a mean±S.E. in absolute units or as percentages of the values obtained immediately before drug administration.

Figure 1A summarizes the changes in mean blood pressure (MBP), heart rate (HR)
and ST segment (S wave height) in the ECG after ET was administered near the coronary ostia in 11 rats. The dose of ET was increased from 0.1 μg/kg to 0.2, 0.5 and 1 μg/kg in a stepwise manner after changes produced by a lower dose subsided. Blood pressure fell temporarily in a dose-related manner. Soon afterwards, blood pressure returned to near control levels at 0.1 and 0.2 μg/kg, but it rose by 17±4% at 0.5 μg/kg. At the highest dose, 1 μg/kg, 9 out of 11 rats died of ventricular fibrillation before blood pressure rose. Heart rate decreased slightly, but in a dose-related manner. The electrocardiogram exhibited bi-directional changes depending on the dose of ET: at lower doses (0.2 and 0.5 μg/kg), the ST segment was depressed, whereas at the highest dose (1.0 μg/kg), it was transiently elevated (from −0.23±0.03 to +0.24±0.06 mV) and was depressed thereafter (to −0.39±0.08 mV). The maximum ST segment elevation coincided with the maximum hypotension.

ET, at doses lower than 0.5 μg/kg, did not elicit arrhythmias. At 0.5 μg/kg, ventricular arrhythmias developed 4.3 to 6.3 min after administration of ET in 9 out of 11 rats, but none of these animals died. At the highest dose, 1 μg/kg, severe ventricular arrhythmias developed in all rats 2–3 min after administration, when blood pressure returned to control levels. Ventricular arrhythmias included ventricular premature contractions (in all rats), ventricular tachycardia (in 10 out
of 11 rats) and ventricular fibrillations (in 9 out of 11 rats), which resulted in death 4.8±0.4 min after injection of ET. Aortic administration of ET produced similar hemodynamic changes (Fig. 1B). However, there were no arrhythmias and no changes in the height of the ST segment.

Figure 2 shows an example of recordings when 1.0 μg/kg of ET was administered into the coronary ostia. In this rat, the ST segment elevation reached a maximum 20 sec after administration of ET (Fig. 2B). At this moment, blood pressure and LVEDP/dtmax reached a minimum and LVEDP rose, suggesting a decrease of myocardial contractile force. After these variables returned to near control values, ventricular tachycardia developed and ventricular fibrillation ensued soon thereafter (Fig. 2C). In a total of 3 rats, LVEDP/dtmax decreased by 34±8% and LVEDP increased by 118±40% at their maxima. These variables returned to near control values at the time when the ventricular arrhythmias developed.

Hemodynamic responses to close-coronary administration of ET were surprisingly similar to those obtained after aortic administration (Fig. 1). The major differences observed following these two administrations lay in the changes in ECG: the close-coronary administration elevated the ST segment and induced ventricular arrhythmias, whereas the aortic administration did not. These results suggest that a fraction of the ET injected near the coronary ostia directly entered the coronary circulation, causing coronary vasoconstriction and affecting the electrical activity of the myocardial cells.

The ST segment elevation together with the decrease of LVP, LVEDP/dtmax and increase of LVEDP indicates the presence of myocardial ischemia, because ET has a direct positive inotropic action in vitro (3-5). Intracoronary administration of ET has been demonstrated to cause an intense coronary vasoconstriction, elevation of the ST segment and decrease of cardiac contractile function in the dog (7-10) and pig (11). The cause of death from intracoronary infusion of ET in the pig has been attributed to cardiac

Fig. 2. An example of cardiohemodynamic recordings before (A) and after (B, C) close-coronary administration of ET at a dose of 1 μg/kg. On the right side are shown recordings at a higher chart speed. Hemodynamic changes reached the maximum in 20 sec (B) and ventricular arrhythmias developed when the S wave, BP, LVEDP and LVEDP/dt returned toward the pre-administration values (C). HR: heart rate, BP: blood pressure, LVP: left ventricular pressure, LVEDP/dt: the first derivative of LVP, ECG: electrocardiogram recorded by the apex-base lead. LVEDP: left ventricular end-diastolic pressure.
insufficiency due to myocardial ischemia (11). In the present study, however, the cause of death was obviously ventricular fibrillation, because a sudden decrease of blood pressure coincided with the development of ventricular fibrillation. ET undoubtedly produced myocardial ischemia as judged by the ST segment elevation and decreased cardiac function. However, the myocardial cells appeared to have recovered from ischemia at the time when ventricular fibrillation developed. Another inducer of coronary spasm, methacholine, produced a much greater ST segment elevation than ET did (+0.46±0.04 mV for methacholine vs. +0.24±0.06 mV for ET, P<0.05 by Student's t-test), but never induced arrhythmias in the present study. Taken together, these results suggest that ventricular fibrillation produced by ET can not solely be attributed to myocardial ischemia, although ischemia is of primary importance. Myocardial ischemia together with accumulation of intracellular Ca²⁺ may account for ET-induced arrhythmias. It is conceivable that ET increases cytosolic Ca²⁺ directly (13) or indirectly through membrane depolarization due to ischemia. A preliminary electrophysiological study using canine Purkinje fibers demonstrated that ET evokes extrasystoles under a hypoxic condition (R. Yorikane et al., unpublished data). In summary, the present study demonstrated that ET is arrhythmogenic. Although myocardial ischemia and subsequent reperfusion are important causal factors contributing to ET-induced arrhythmias, relatively small elevation of the ST segment and the little decrease of cardiac function caused by ET suggest that other mechanisms may underlie these arrhythmias as well. Obviously, further studies are needed to elucidate the mechanisms responsible for ET-induced arrhythmias. However, whatever the mechanisms may be, the arrhythmogenic action of ET is important in considering the role of ET in the pathogenesis of cardiac sudden death due to coronary spasm, particularly in a situation where a large amount of ET is locally released in the coronary vascular bed.

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