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Short communication

Diltiazem, an antagonist of calcium channels, reduces norepinephrine in the rat myocardium

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Abstract

The present study examined the effect of diltiazem, a calcium channel antagonist, on potassium (K^+) depolarization-induced norepinephrine (NE) release in rat myocardium using a flexibly mounted microdialysis technique. When high concentration of KCl (70 mM) was directly infused in rat heart through a microdialysis probe, the level of NE gradually increased in a time dependent manner. However, when a high concentration of KCl (70 mM) was administered to diltiazem-pretreated animals, it failed to increase the KCl-induced dialysate NE. These results suggest that calcium (Ca^{2+}) may play a key role in K^+ depolarization-induced NE release in the myocardium.
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Keywords: Potassium depolarization; Norepinephrine; Diltiazem; Microdialysis

1. Introduction

Although the role of catecholamines on myocardial cellular injury is unclear, high concentrations of catecholamines can damage the myocardium [1]. In experimental animals, infusion of a large dose of catecholamine, epinephrine, norepinephrine (NE) and isoproterenol produces a lesion that is histologically similar to myocardial infarction [2]. It is well known that NE release is increased by potassium (K^+) depolarization [3,4]. Numerous investigators have reported that ischemia also increases NE levels [5–7]. The release of intracellular calcium (Ca^{2+}) by NE may make its stimulated Ca^{2+} influx less susceptible to inhibition by diltiazem, a calcium channel antagonist [8,9]. Ca^{2+} ions might be a very important factor in induction of irreversible ischemic injury [10,11]. Therefore, it is important to explain the relationship between Ca^{2+} channels and NE release in some disease states.

The present study examined the effect of diltiazem, a calcium channel antagonist, on K^+ depolarization-induced NE.

2. Experimental

2.1. Animals

Adult Male Wistar rats weighing 300–400 g were kept in an environmentally controlled room (20–23 °C, 50–60% humidity, illuminated from 7:00 to 19:00 h) and fed with food and water ad libitum. The rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and prepared for cardiac microdialysis heart perfusion by a method previously shown [5]. This study was approved by the Ethical Committee for Animal Experiments of Oita Medical University, Japan.

2.2. Experimental protocol

Diltiazem (Herbesser; Tanabe Seiyaku Co. Ltd., Japan) was continuously administered by intravenous infusion. In the preliminary experiments, the relative recovery of 10^{-6} M standard solution of NE was 17.0 ± 0.7%, at a flow rate of 1 μl/min. Details of the flexibly mounted microdialysis technique (O-system) [5] and its application to measure biological substances in the interstitial space have been described previously. We created a suitable microdialysis probe. Dialysis probe was implanted from the epicardial surface

Abbreviations: NE, norepinephrine; HPLC-EC, a high performance liquid chromatographic-electrochemical.

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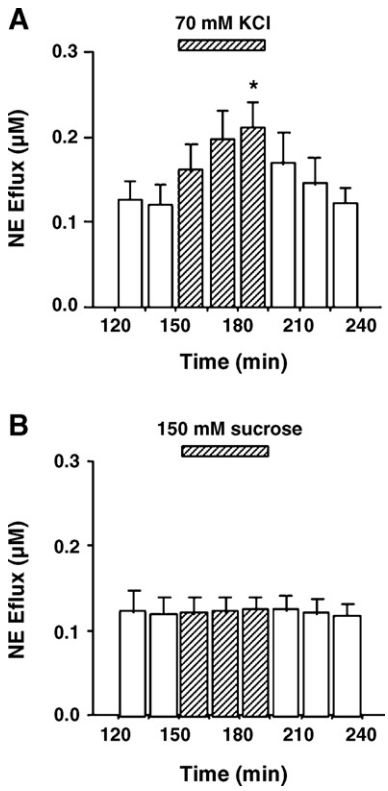

Fig. 1. Changes in the levels of NE in the dialysate after high KCl treatment. After a 120-min washout with Ringer's solution, the time course of dialysate NE in extracellular fluid of myocardium after KCl (diagonally shaded column) treatment was investigated. When KCl (70 mM; A) and sucrose (150 mM; B) at 150 min after probe implantation were infused directly through microdialysis probe in rat heart for 45 min, dialysate samples were collected at 15-min intervals and immediately assayed for NE using an HPLC-EC procedure. Values are mean±S.E.M. for six animals; *$P<0.05$ vs. pre-drug value.

into left ventricular myocardium to the depth of 3 mm. The synchronized movement of the tip of the microdialysis probe with the beating ventricle minimized the tissue injury that would otherwise be caused by friction between the probe and the muscle tissue. The probe was perfused with Ringer's solution containing 147 mM NaCl, 2.3 mM $CaCl_2$ and 4 mM KCl (pH 7.4).

2.3. Analytical procedure

The dialysate samples were immediately injected for analysis into a high performance liquid chromatographic-electrochemical (HPLC-EC) system equipped with a glassy carbon working electrode (Eicom, Kyoto, Japan) and an analytic reverse-phase column on an Eicompak MA 5ODS column (5 μm 4.6×150 mm; Eicom). The working electrode was set at a detector potential of 0.75 V. Each liter of mobile phase contained 1.5 g 1-heptansulfonic acid sodium salt (Sigma), 0.1 g Na_2EDTA, 3 ml triethylamine (Wako Pure Chemical Industries, Japan) and 125 ml acetonitrile (Wako) dissolved in H_2O. The pH of the solution was adjusted to 2.8 with 3 ml phosphoric acid (Wako). The results were reported as means± SE output.

2.4. Statistical analysis

All values are presented as means±SE. The significance was determined by using ANOVA with Fisher's post hoc test. A P value of less than 0.05 was regarded as being statistically significant.

3. Results and discussion

In an in vivo perfusion system, the NE were monitored in the dialysates from a rat heart. The dialysis probe was perfused with Ringer's solution throughout the experiment. After probe implantation, dialysate NE gradually decreased, reaching an almost steady-state level at 135–150 min after probe implantation. Therefore, after obtaining two control fractions (120–135 and 135–150 min), KCl was directly infused in the rat heart through a microdialysis probe. The high concentration of KCl (70 mM) significantly increased the level of NE (Fig. 1A). Equivalent increases in osmotic concentration of the Ringer's solution, made by adding sucrose (150 mM), did not affect the dialysate NE concentration (Fig. 1B). However, when similar experiments were performed with various concentrations of diltiazem (5, 10 and 50 mg/kg) and systemic administration of i.v. injection, the dialysate NE reduced in a concentration-dependent manner over the concentration range of 5–50 mg/kg; the maximum effect, was 28.9±7.1% of control (Fig. 2). The occurrence of intracellular Ca^{2+} plays an important role in cardiac dysfunction. It is known that KCl-induced depolarization enhances Ca^{2+} overload [12,13]. However, the exact mechanisms of loading to the development of this abnormality are far from clear. Intracellular calcium overload has been reported to be one of the causes of ischemia–reperfusion injury [14]. We previously reported that ischemia–reperfusion of

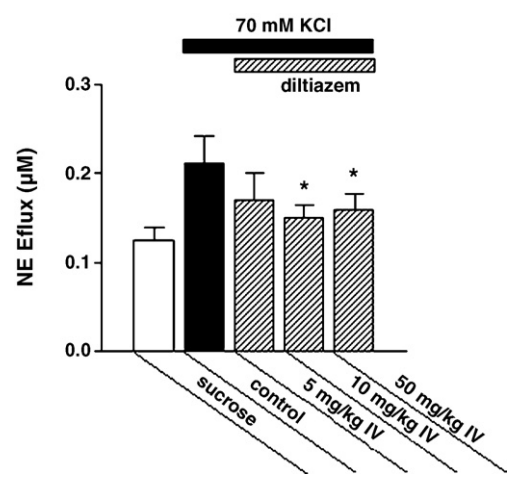

Fig. 2. Effect of diltiazem on NE efflux by high KCl. Equivalent increases in osmotic concentration of the Ringer's solution, made by adding sucrose (150 mM; diagonally shaded column) which was administered to the rat. When high KCl (70 mM; closed column) was administered to diltiazem (5, 10 and 50 mg/kg i.v.) -pretreated animals (closed column), the dialysate NE were compared with the nontreated group (control; open column). Dialysate samples were collected at 15-min intervals and immediately assayed for NE using an HPLC-EC procedure. Values are mean±S.E.M. for six animals; *$P<0.05$ vs. control group.

myocardium significantly increases the levels of NE [5]. This finding is in agreement with our data that K^+ depolarization induces NE release. These results suggest that Ca^{2+} may play a key role in depolarization-induced NE release in the myocardium. However, further experiment is necessary to confirm the relation between Ca^{2+} overload and NE release.

In conclusion, I have found that diltiazem decreases NE release by KCl-induced depolarization. Consequently, the inhibition of Ca^{2+} channel may decrease the NE release of a rat ventricular muscle in situ. These experiments in cardiac microdialysis have versatile applications and offer new possibilities for in vivo study in the pathogenesis of heart disorders.

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