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ORIGINAL ARTICLE

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Protective effects of calcitonin gene-related peptide-mediated evodiamine on guinea-pig cardiac anaphylaxis

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Abstract Previous investigations have indicated that the pharmacological effects of evodiamine, a major alkaloidal component of the dried, unripe fruit of *Evodia rutaecarpa* Bentham (Rutaceae), are associated with stimulation of calcitonin gene-related peptide (CGRP) release and CGRP prevents cardiac anaphylactic injury. In the present study, the protective effects of evodiamine on cardiac anaphylaxis were examined. Presensitized guinea-pig hearts challenged with specific antigen (bovine serum albumin) caused a marked decrease in coronary flow, left ventricular pressure and its derivatives ($\pm dp/dt_{max}$), an increase in heart rate, and prolongation of P-R interval. Evodiamine (0.3 μM or 1 μM) markedly increased the content of CGRP in the coronary effluent concomitantly with a significant improvement of cardiac function and alleviation of the extension of P-R interval. Evodiamine at the concentration of 1 μM also inhibited the sinus tachycardia. The protective effect of evodiamine on cardiac anaphylaxis was abolished by $CGRP_{8-37}$, the selective CGRP receptor antagonist. These results suggest that evodiamine possesses a protective effect of cardiac anaphylactic injury and that the effect of evodiamine is related to stimulation of CGRP release.

Keywords Cardiac anaphylaxis · Calcitonin gene-related peptide · Evodiamine · Guinea-pig

Introduction

Cardiac anaphylaxis is a laboratory model of clinically recognized immediate hypersensitive reactions affecting the heart. In the isolated guinea pig heart model, anaphylaxis is an acute dysfunction characterized by tachycardia, arrhythmias, and coronary vasoconstriction. It has been reported that multiple mediators are involved in cardiac anaphylaxis and calcitonin gene-related peptide (CGRP) released during cardiac anaphylaxis may function as a mitigating factor against threatening vasoconstricting mediators (Rubin and Levi 1995; Schuligoi et al. 1997). Our previous studies have shown that stimulation of endogenous CGRP release or exogenous administration of CGRP alleviates cardiac anaphylactic injury (Dai et al. 2000; Xiao et al. 2001).

Evodiamine, a major alkaloidal principle of *Evodia* fruits, was isolated from the dry unripe fruit of *Evodia rutaecarpa* Bentham (Rutaceae) (Jeng et al. 1995). It has been reported that evodiamine has a wide variety of pharmacological activities, such as contractile response of the bronchus (Kobayashi et al. 2000), antianoxic action (Yamahara et al. 1989), antinociceptive effects (Matsuda et al. 1997), inhibition of platelet aggregation (Kobayashi et al. 2001a) and vasorelaxation (Chiou et al. 1992, 1996). Previous studies have shown that bronchoconstrictive and anti-obese effects of evodiamine are related to the activation of vanilloid receptors (Kobayashi et al. 2000, 2001b). More recently, the positive inotropic and chronotropic effects of evodiamine were attributed to their interaction with vanilloid receptors and the resultant release of CGRP in the guinea pig isolated left atria (Kobayashi et al. 2001a). According to the release of CGRP stimulated by evodiamine and the protective effects of CGRP on cardiac anaphylactic injury, in the present study, therefore, we examined the protective effects of evodiamine on cardiac anaphylaxis and also explored whether the protective effects of evodiamine are related to stimulation of endogenous CGRP release.

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Materials and methods

Isolated perfused heart. Guinea pigs of both sexes weighing 280–400 g were anesthetized with sodium pentobarbital (40 mg·kg^{-1}, i.p.). Hearts were rapidly excised and then placed in a Langendorff

apparatus maintained at 37°C. The heart was perfused retrogradely with Krebs-Henseleit (K-H) buffer saturated with 95% O_2 and 5% CO_2, at a constant perfusion pressure of 100 cm H_2O. The K-H buffer had the following composition (in mM): NaCl 119.0, $NaHCO_3$ 25.5, KCl 4.3, KH_2PO4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, and glucose 11.0. A partially inflated fluid filled balloon attached to a pressure transducer was placed in the left ventricle through the mitral valve. The left ventricular pressure (LVP) and its first derivatives ($\pm dp/dt_{max}$), and heart rate (HR) were continuously monitored. The resulting electrical signals were digitized by a MacLab analogue-to-digital converter and recorded on a Power Macintosh 7220 computer. Bipolar surface electrocardiograms were recorded from electrodes placed on the right atrium and apex, and the P-R interval was calculated to examine the degree of atrioventricular nodal conduction block in cardiac anaphylaxis. Coronary flow (CF) was measured by timed collection of coronary effluent.

Isolated heart anaphylaxis. Guinea pigs were actively sensitized by intraperitoneally injection of the three successive doses of 20 mg bovine serum albumin in 0.5 ml saline every alternate day. Three weeks after the last injection, the hearts of presensitized guinea pigs were isolated and perfused as indicated above and eventually challenged intra-aortically with 5 mg bovine serum albumin in 0.2 ml K-H buffer.

Calcitonin gene-related peptide assay. The whole coronary effluent of perfusion fraction was collected for 5 min before and after treatment with evodiamine or vehicle, and acetic acid (final concentration 0.2 mM) was added. The samples were desalted using Sep-Pak C_{18} cartridge, lyophilized and stored at –20°C until assay. Radioimmunological assay (Zaidi et al. 1990) was used to measure CGRP-like immunoreactivity (CGRP-LI) in the perfusion fractions by using immunoreactivity kits with antisera raised rat CGRP, ^{125}I-labelled CGRP and rat CGRP standard.

Histamine assay. Samples of coronary effluent were collected for 1 min in the first 1-min period after antigen challenge. The histamine content of coronary effluent was determined by a manual fluorometric method previously described (Shore et al. 1959).

Reagents. Evodiamine (purity: >99%) was obtained from School of Pharmaceutical Sciences, Central South University (Changsha, China). Bovine serum albumin, dimethyl sulfoxide (DMSO), $CGRP_{8-37}$ and histamine were purchased from Sigma (St. Louis, MO, U.S.A). Evodiamine and $CGRP_{8-37}$ were initially dissolved in DMSO and distilled water, respectively, and further diluted in K-H buffer to proper final concentration. The final concentration of DMSO in the solution did not exceed 0.1%. Bovine serum albumin was dissolved in K-H buffer. Radioimmunoassay kits for measurement of CGRP were obtained from Dong-Ya Immunity Technology Institution (Beijing, P.R. China).

Experimental protocols. All hearts had an initial stabilization period for 20–30 min with K-H buffers. In the control group, presen-

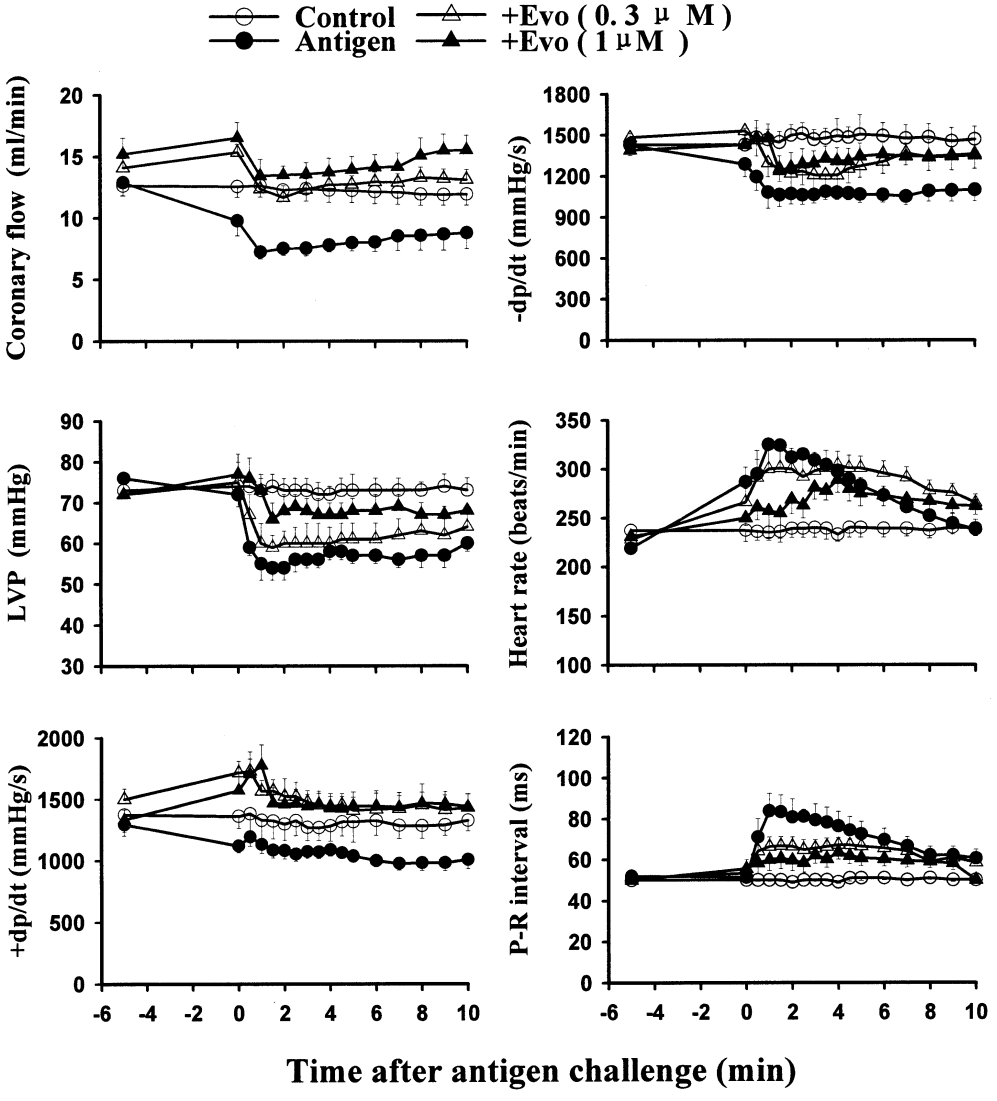

Fig. 1 Effect of evodiamine on cardiac dysfunction in sensitized guinea pigs. *Evo* evodiamine (0.3 or 1 μM). The heart was perfused with evodiamine for 5 min before antigen challenge, and then the drug remained in the perfusion throughout the remainder of the experiment. Values are means ± SEM (n=6–9)

sitized hearts were treated with 0.2 ml warm K-H buffer. In the case of antigen challenge, presensitized hearts were challenged intra-aortically with 5 mg of bovine serum albumin in 0.2 ml warm K-H buffer. For evodiamine, presensitized hearts were perfused with evodiamine (0.3 or 1 μM) for 5 min before antigen challenge, and the drug remained in the perfusate for the remainder of the study. For the studies on the effect of $CGRP_{8-37}$ on cardioprotection afforded by evodiamine, presensitized hearts were perfused with $CGRP_{8-37}$ (0.3 μM) for 5 min and then perfused with evodiamine (1 μM) in the presence of $CGRP_{8-37}$.

Statistical analysis. Data were expressed as means ± SEM. Statistical evaluation was performed with ANOVA and Student-Newman-Keuls *t*-tests. The significance level was chosen as $P<0.05$.

Results

There were no changes in cardiac function and histamine in the control group. Antigen challenge of spontaneously beating presensitized guinea pig hearts caused sinus tachycardia, atrioventricular nodal conduction block and a decrease of CF, LVP, and $\pm dp/dt_{max}$. Evodiamine at a concentration of 0.3 or 1 μM significantly improved cardiac function, as shown by increasing CF, LVP and $\pm dp/dt_{max}$. Evodiamine at the concentration of 1.0 μM also alleviated the extension of P-R interval, while at lower concentrations (0.3 μM), evodiamine had no effect on the atrioventricular block induced by antigen challenge (Fig. 1). However, the protective effects of evodiamine were abolished in the presence of $CGRP_{8-37}$, the selective CGRP receptor antagonist (Fig. 2).

Evodiamine at the concentration of 0.3 or 1 μM caused a marked increase in the release of CGRP-LI. In this case the release of CGRP-LI stimulated by evodiamine at higher concentrations (1.0 μM) was significantly greater compared to evodiamine at a concentration of 0.3 μM ($P<0.01$) (Fig. 3).

Antigen challenge produced a significant increase in the release of histamine (Fig. 4). However, evodiamine did not affect the increased released of histamine induced by antigen challenge (Fig. 4).

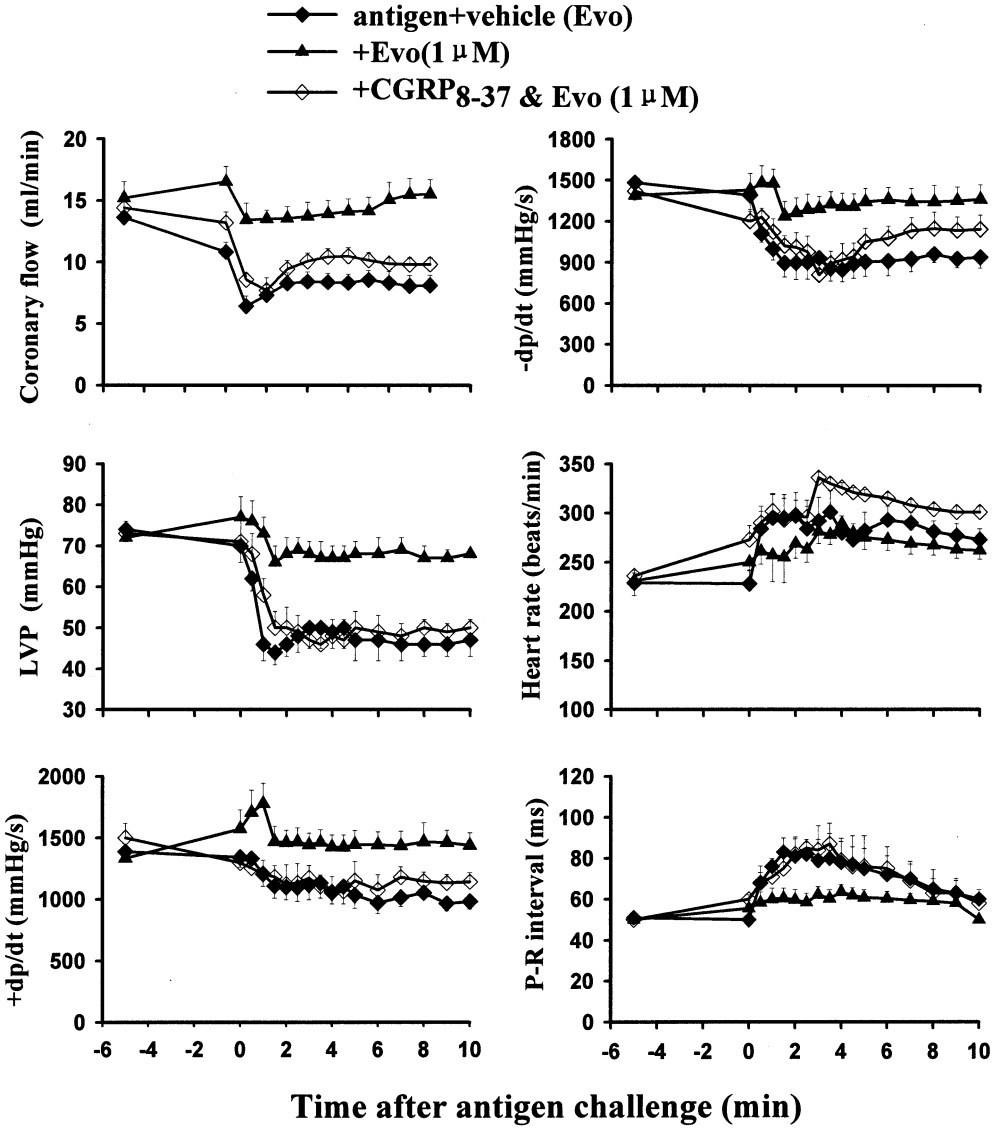

Fig. 2 Effect of $CGRP_{8-37}$ on cardioprotection of evodiamine. The heart was perfused with $CGRP_{8-37}$ for 5 min and then perfused with evodiamine in the presence of $CGRP_{8-37}$. Values are means ± SEM (n=6–9)

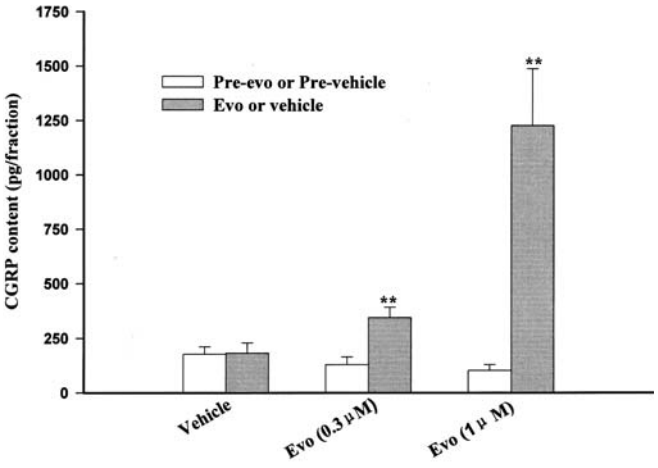

Fig. 3 Effect of evodiamine on the release of CGRP. The coronary effluent of perfusion fraction (5 min) was collected in the absence or presence of drugs. *Evo* evodiamine. Values are means ± SEM, n=6–7. $^{**}P$<0.01, compared with pre-evo or pre-vehicle

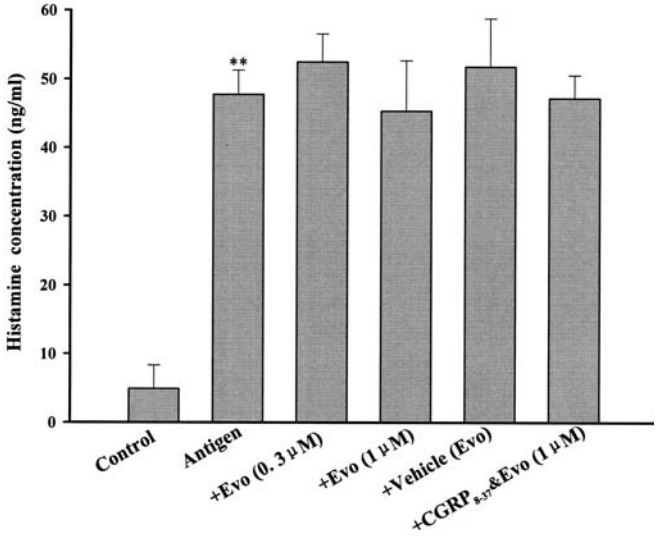

Fig. 4 Effect of evodiamine on the release of histamine. The coronary effluent was collected in the first 1-min period after antigen challenge. Antigen challenge was performed in all experiments, except the controls. *Evo* evodiamine; vehicle: 0.1% DMSO. Values are means ± SEM, n=6–9. $^{**}P$<0.01, compared with control

Discussion

The previous investigations have suggested that the heart reacts as a target organ in systemic anaphylaxis (Capurro and Levi 1975), and cardiac anaphylaxis has been ascribed to stimulation of endogenous mediators (Hellar and Regal 1988; Cooper 1993; Mest et al. 1995). Histamine, leukotrienes, platelet-activating factor, adenosine, and thromboxane were considered as contributors to cardiac anaphylactic injury (Wolff and Levi 1986; Marone et al. 1995; Yaacob and Piper 1988; Mest et al. 1995; Hughes et al. 1984), and some coronary vasodilators such as NO, bradykinin and CGRP may be beneficial to cardiac anaphylaxis reaction (Rubin and Levi 1995; Abend et al. 1996; Schuligoi et al. 1997).

It has been shown that CGRP, a principal neurotransmitter in sensory nerves, is distributed widely in cardiovascular tissues (Gibson et al. 1984; Wharton et al. 1986). CGRP is the most potent vasodilator (Yallampalli et al. 2002). CGRP, besides relaxing vascular smooth muscle, has a protective effect on the myocardium and endothelial cells. It has been reported that exogenous CGRP prevents the myocardial or endothelial injury induced by ischemia reperfusion, adriamycin, lysophosphatidylcholine, endothelin, or oxygen free radicals (Li et al. 1996; Yang et al. 1996; Peng et al. 1996, 1998; Tao et al. 1997; Zhang et al. 1994). Our recent work has shown that ischaemic preconditioning, or acute application of capsaicin, or treatment with nitroglycerin to stimulate the release of endogenous CGRP also significantly attenuates cardiac anaphylactic injury (Dai et al. 2000; Xiao et al. 2001).

Recently, it has been shown that evodiamine has an antinociceptive effect, inhibition of platelet aggregation and vasodilator effects, capsaicin-like anti-obese activities, and the pharmacological effects of evodiamine is similar to that of capsaicin which activates vanilloid receptors (Kobayashi et al. 2000, 2001b). There is evidence that evodiamine produces the positive inotropic and chronotropic effects via activating vanilloid receptors to evoke release of CGRP from capsaicin-sensitive nerves in the isolated guinea pig left atria (Kobayashi et al. 2001a). Our recent study has indicated that rutaecarpine, another alkaloidal component of the dried, unripe fruit of *Evodia rutaecarpa* Bentham (Rutaceae), attenuated ischaemic myocardial injury in the isolated guinea pig heart and the effect of rutaecarpine is also related to stimulation of endogenous CGRP release via activating vanilloid receptors (Hu et al. 2002). In the present study, evodiamine significantly reduced antigen–challenged cardiac dysfunction, as shown by increasing left ventricular pressure, its derivatives ($\pm dp/dt_{max}$) and coronary flow, and the cardioprotection afforded by evodiamine was abolished by $CGRP_{8–37}$, suggesting that the protective effect of evodiamine on cardiac anaphylactic injury is also mediated by endogenous CGRP.

Histamine is considered to be one of the major mediators mediating the inflammation in cardiac anaphylaxis. It has been reported that H_1 receptors mediate coronary vasoconstriction and cardiac depression (Felix et al. 1998, Tucker et al. 1975), whereas H_2 receptor agonists produce coronary and systemic vasodilation as well as increases in heart rate (HR) and ventricular contractility (Tucker et al. 1975). Histamine H_3 receptors have recently been identified on presynaptic terminals of sympathetic effector nerves that innervate the heart and systemic vasculature (Endou et al. 1994; McLeod et al. 1993), and H_3 receptor activation accentuates the degree of anaphylactic shock via inhibiting endogenous norepinephrine release from sympathetic nerve (Endou et al. 1994). The results of the present study confirmed previous observation that antigen challenge caused a significant increase in the release of histamine (Wolff and Levi 1986; Marone et al. 1995). How-

ever, in the present study, evodiamine had no effect on the increased release of histamine by antigen challenge. Our previous work also showed that exogenous CGRP, or nitroglycerin or ischemiac preconditioning to evoke the release of CGRP from cardiac sensory nerves did not affect the increased release of histamine by antigen challenge in the isolated guinea-pig hearts. These results support the conclusion that CGRP, endogenous or exogenous, does not affect the release of histamine by antigen challenge. Previous investigations have shown that CGRP has a direct protection of myocytes (Ren et al.1993). It is probable that CGRP opposes myocardial injury induced by some anaphylactic mediators via some non-specified ways.

In conclusions, the present study suggests that evodiamine prevents antigen-challenged cardiac dysfunction. The present result also suggests that the protective effect of evodiamine is related to stimulation of endogenous CGRP release.

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