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Endocardial endothelium modulates cardiac responses to histamine and impromidine in isolated working right ventricle of guinea pigs[1]

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ABSTRACT In isolated working right ventricle of guinea pigs, the hypothesis that endocardial endothelium (EE) might be involved in the modulation of cardiac responses to histamine receptor agonists was tested. The functional EE was denuded by switching Krebs perfusion solution to the solution containing saponin (30 $\mu g \cdot ml^{-1}$) for 2 min at a rate of 16 ml $\cdot min^{-1}$, followed by thorough washing with Krebs solution. The cardiac responses to histamine receptor agonists were compared in the presence and absence of EE. Bolus injection of histamine 0.5 mg into right ventricle elevated the right ventricular pressure (RVP), $+dP/dt_{max}$, and $-dP/dt_{max}$ by 11%, 17%, and 35%, respectively, in the presence of intact EE; whereas by 30%, 43%, and 92%, respectively, after chemically selective denudation of EE with saponin. Similarly, impromidine (a H_2 receptor agonist) 1, 3, 9, 27, and 54 μg obviously potentiated the RVP and $\pm dP/dt_{max}$ in a concentration-dependent manner in preparations either with or without EE. The effects, however, could be greatly enhanced in the absence of EE. Pulmonary outflow was declined at 27 μg impromidine in EE-removed group. The results suggested that the augmentation of cardiac responses produced by histamine receptor agonists in EE-denuded preparations might be due to blockade of release of endothelium-derived relaxing factor, resulting in a reduced abbreviating effect on the myocardial contraction.

KEY WORDS endocardium; histamine; impromidine; myocardial contraction; heart ventricle

We have recently demonstrated that endocardial endothelium (EE) modulates the magnitude and characteristics of the peak contractile performance of isolated guinea pig papillary muscles[1]. The actions of several vasoactive substances on myocardial performance have also been reported to be mediated or modulated via EE[2]. In vascular preparations, histamine induces vasoconstriction in the absence of endothelium but relaxation in the presence of endothelium by releasing endothelium-derived relaxing factor[3]. Ontogenetically similar to vascular endothelium, EE consists of a monolayer of closely apposed cells lining the internal surface of the heart. It is not certain, however, whether EE can also modulate the inotropic responses to histamine receptor agonists. The present study was to investigate the possible role of EE in the cardiac responses to histamine receptor agonists by comparing their effects in the presence and absence of intact EE.

MATERIALS AND METHODS

Drugs Saponin (E Merck, USA), histamine (Sigma, USA), and impromidine (SK & F Labs, UK). All drugs were initially dissolved in Krebs solution and further diluted in Krebs solution to the desired final concentrations.

Preparation of isolated working right ventricle The isolated working right ventricle of guinea pig has been achieved successfully in our laboratory. The hearts of guinea pigs weighing 315 ± *s* 30 g were isolated and prepared as described by Zhang *et al*[4] with some modifications. Briefly, the guinea pig was stunned and the chest opened immediately to expose the heart. The aorta was quickly cannulated and perfused retrogradely with 37 C Krebs solution at a rate of 8 $ml \cdot min^{-1}$. Under this condition, both hili were ligated and the lungs excised. The superior vena cava

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was inserted with a cannula through which a small plastic catheter (ID 1 mm) was introduced into the right ventricle to measure the intraventricular pressure. The pulmonary artery was cannulated and the inferior vena cava was ligated. The heart was connected to the perfusion apparatus, while a constant retrograde perfusion was maintained via the aorta. The right atrial cannula, which was connected to a right perfusion reservoir, was opened to initiate the working right heart. The right atrial filling pressure was set at 0.29 kPa and the pulmonary artery load at 0.92 kPa before starting the experiment. The Krebs solution was saturated with 95% O_2 + 5% CO_2, and kept at 37 ± 0.5℃, pH 7.4 ± 0.5. It consisted of: NaCl 118; KCl 5.4; $CaCl_2$ 2.0; $MgSO_4 \cdot 7H_2O$ 1.2; $NaHCO_3$ 25; NaH_2PO_4 1.0, and glucose 11.1 mmol·L^{-1}.

Experimental protocol The preparations were equilibrated for 15 min. Parallel experiments were performed in preparations with or without EE. Saponin has been shown at a suitable concentration to cause endothelium denudation without altering the underlying muscle integrity and receptor function[5,6]. Signal transduction and contractile responses to various vasoactive substances were preserved with this chemical denudation[7]. Thus, half of the isolated working right ventricles were randomly selected for chemical denudation of functional EE by switching the perfusion solution to the solution containing saponin (30 μg·ml^{-1}) for 2 min at a rate of 16 ml·min^{-1}, followed by thorough washing with Krebs solution.

Histamine (0.5 mg) or impromidine (1, 3, 9, 27, and 54 μg) in 0.5 ml Krebs solution was injected in bolus through an inlet in the right atrial cannula within 10 s. After drug administration, the pulmonary effluent within the first 2 min was discarded. The perfusion pressure was monitored by a pressure transducer and the following parameters were measured with a polygraph (RM-6000, Kohden, Japan): right ventricular pressure (RVP) and its dP/dt, right ventricular end-diastolic pressure (RVEDP), aortic perfusion pressure (APP). The pulmonary outflow (PF) was simultaneously recorded on an electromagnetic flowmeter (Kohden, Japan). One platinum wire electrode was placed respectively on the top and the bottom of the heart to record the electrocardiogram.

Statistical analysis Results were expressed as $\bar{x} \pm s$. Statistical differences between the 2 methods were compared by t test.

RESULTS

Effects of EE on inotropic responses to histamine Exposure to saponin-containing solution (30—50 μg·ml^{-1}) for 2—3 min did not alter the steady-state cardiac performance of isolated working right ventricle when normal perfusion was restored (Tab 1). Thus,

Tab 1. Cardiac performances of isolated working right ventricles before and after saponin treatment (30 μg·ml^{-1} for 2 min), $n=5-6$, $\bar{x} \pm s$, * $P>0.05$ *vs* Before. Individual comparison of t test was used.

Parameters	Before	After
Right ventricular pressure, kPa	2.8±0.6	2.9±0.4*
$+dP/dt_{max}$, kPa·s^{-1}	40±10	39±14*
$-dP/dt_{max}$, kPa·s^{-1}	24±9	24±11*
Pulmonary outflow, ml·min^{-1}	46±9	45±8*
Heart rate, bpm	180±25	185±36*
Aortic perfusion pressure, kPa	6.7±2.7	7.6±2.1*

the preparations were perfused with saponin (30 μg·ml^{-1}) for 2 min to denude the EE. When observing the effects of a single concentration of histamine (0.5 mg in 0.5 ml Krebs solution), the elevations in RVP and $\pm dP/dt_{max}$ reached their maximum 2—3 min after histamine injection and returned to normal about 15 min later, in both groups. After saponin treatment, there was a marked augmentation of inotropic responses to histamine in preparations without EE (Tab 2). In contrast, RVEDP, HR, and PF remained unaltered in comparison to those in the histamine group in the presence of intact EE.

Effects of EE on cardiac responses to impromidine Bolus injection of impromidine 1, 3, 9, 27, and 54 μg obviously elevated the RVP and $\pm dP/dt_{max}$ in a concentration-dependent manner (Fig 1). Denudation of EE potentiated the cardiac responses to impromi-

Tab 2. Myocardial contraction mediated by bolus injection of 0.5 mg histamine in isolated working right ventricles with/without endocardial endothelium (EE). $n=5$, $\bar{x}\pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* with EE.

Parameters	with EE	without EE
RVP, kPa	3.1±0.2	4.0±0.3**
$+dP/dt_{max}$, kPa·s^{-1}	46±5	57±7**
$-dP/dt_{max}$, kPa·s^{-1}	32±3	46±6***
RVEDP, kPa	0.21±0.04	0.22±0.05
Heart rate, bpm	207±10	218±18*
Pulmonary outflow, ml·min^{-1}	48±13	49±9*

RVP: right ventricular pressure; $\pm dP/dt_{max}$: maximal rate of rise of RVP; RVEDP: right ventricular end-diastolic pressure.

dine, resulting in increments in RVP and $\pm dP/dt_{max}$, comparing to the preparations with intact EE. However, the augmentation of inotropic action can be completely abolished by

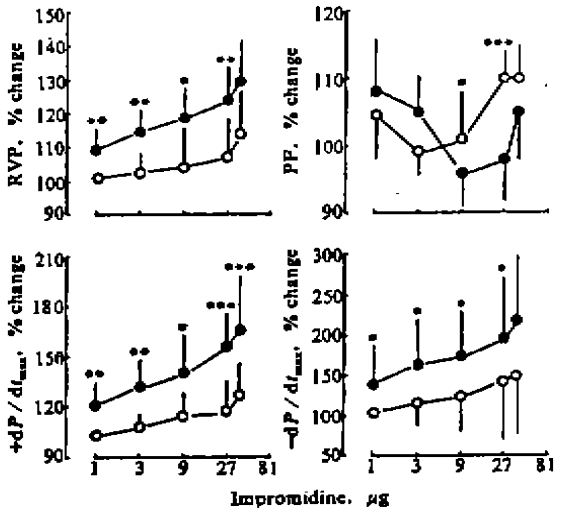

Fig 1. Effect of impromidine on cardiac performance of isolated working right ventricle of guinea pigs with (○) or without (●) endocardial endothelium. PVP: right ventricular pressure; PF: pulmonary outflow; $\pm dP/dt_{max}$: maximal rate of the rise of PVP. $n=5$, $\bar{x}\pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* with endothelium.

bolus injection of 0.1 mg famotidine, a H_2 receptor specific blockader. PF decreased initially, and then increased obviously in both groups. In EE-removal group, it was declined at 27 μg impromidine (Fig 1). Impromidine mediated the positive chronotropic effect, which was enhanced by EE denudation. APP remained stable at 6.0—6.7 Pa in both preparations during the entire course of the experiment. With increasing concentration of impromidine up to 54 μg, RVEDP increased from the basal level 66.6 to 186.6 Pa in the EE-intact group, and from 53.3 to 239.9 Pa in EE-denuded group. No significant discrepancy between the two preparations was identified at any concentration of impromidine studied.

DISCUSSION

It was previously reported that vasodilator response to histamine was endothelium-dependent in most vascular preparations[1-8]. In the present study, we examined the effects of EE on inotropic responses to exogenous histamine receptor agonists. The cardiac responses produced by histamine or impromidine were obviously potentiated after denudation of EE with saponin treatment in isolated working right ventricle of guinea pigs, indicating that the augumentation of cardiac responses was endocardium-related.

The isolated working right ventricle preparation was employed in the present study. In this model, only EE was removed by brief perfusion with Krebs solution containing low concentration of saponin, which excluded the possibility of the involvement of coronary vascular endothelium in Langendorff or isolated working left ventricle preparations.

The heart occupies a unique position in the circulation allowing intimate contact with and exchange of substances present in the circulating plasma[9]. However, consideration has only recently been directed to the influ-

ence of EE in regulating the contractile performance of the underlying myocardium. Indeed, a novel unidentified agent, provisionally named 'endocardin' has been shown to be released from EE[10]. Endocardin possesses a unique prolonging effect on myocardial contraction. In contrast, endothelium-derived relaxing factor released from EE elicits an opposite effect of abbreviating the contraction. It has well been documented that histamine induces relaxation in vascular preparations with intact endothelium by releasing endothelium-derived relaxing factor. Thus, it was likely that stimulation or activation of EE by histamine receptor agonists might result in the production of endothelium-derived relaxing factor or/and endocardin, which, in turn, acted as a modulator of the cardiac performance.

In conclusion, our findings clearly indicated that cardiac responses to histamine receptor agonists can be greatly enhanced by denudation of EE in isolated working right ventricle preparations. We recently reported that EE can modulate the myocardial contractile performance in isolated guinea pig papillary muscles. Hence, the data presented here extended our previous findings to the isolated working heart preparations and further supported the hypothesis that EE might play an important role in the modulation of the performance of subjacent myocardium in mammalian hearts.

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心内膜调控组胺受体激动剂对离体豚鼠右心工作心脏的效应

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摘要 在离体豚鼠右心工作心脏，单次剂量组胺(0.5 mg)注入右心室使 RVP，$\pm dP/dt_{max}$ 分别增加11%，17%和35%，而预先用皂甙(30 $\mu g \cdot ml^{-1}$)选择性去除右心心内膜后则分别增加30%，43%和92%. 同样，双咪硫胍可剂量依赖性地增强心肌收缩且该效应可被去除心内膜而大大加强. 结果表明心内膜参与调控组胺受体激动剂的心脏效应.

关键词 心内膜；组胺；双咪硫胍；心肌收缩；心室