

Influence of ramipril on release of norepinephrine during sympathetic nerve stimulation in isolated rabbit hearts

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ABSTRACT The present study was performed to determine norepinephrine (NE) release during sympathetic nerve stimulation (SNS) and the inhibitory action of ramipril on the release of NE induced by the stimulus in isolated perfused rabbit hearts. SNS resulted in increases of NE ranged from 2.8 ± 0.9 ng/ml to 10.2 ± 2.7 ng/ml. The addition of ramipril 10 μ g/min to the perfusate for 30 min significantly reduced NE release ($p < 0.05-0.01$). The results suggest that ramipril as an inhibitor of angiotensin converting enzyme (ACEI) may be involved in the angiotensin II-mediated facilitation of adrenergic neurotransmission.

KEY WORDS ramipril; norepinephrine; sympathetic nervous system; kininase II; isolated heart

Angiotensin converting enzyme inhibitors (ACEI) have been introduced clinically as antihypertensive drugs. However, the antihypertensive mechanism of ACEI cannot be solely explained by an inhibition of the

plasma renin-angiotensin system (RAS) with reduced vasoconstrictor action of the circulating angiotensin II. Inhibition of kininase II with an increase of endogenous bradykinin did not appear to play the major role. Ramipril (Hoe 498) is an active ACEI⁽¹⁾. It has potent antihypertensive effects on many animal hypertension models including SHR⁽²⁾. Our previous study have been reported that ACEI attenuated the effects of sympathetic nerve stimulation (SNS) on the heart⁽³⁾. In this study, the question whether the antihypertensive effects of ACEI is related to the influence of the release of sympathetic neurotransmitter (NE) was investigated. We anticipate that the interference of ACEI on NE release may play an important role in their antihypertensive effect.

MATERIALS AND METHODS

New Zealand rabbits of either sex

weighing $2.5 \pm \text{SD } 0.5$ kg were killed by a blow on the head and exsanguination from the carotid artery. The hearts with right sympathetic nerve were isolated and prepared according to the modified method of Langendorff⁽⁴⁾. The perfusion pressure of Krebs-Henseleit solution⁽¹⁾ was maintained at 7.36 kPa (75 cm H₂O, $37 \pm 2^\circ\text{C}$). A period of 20–30 min was allowed for maintaining the equilibrium of the perfused heart. The preparations were subject to a constant resting tension of 5 g. Force of contraction (FC), coronary flow (CF) and heart rate (HR) were recorded as described previously⁽³⁾.

The cardiac sympathetic nerves were stimulated twice for 1 min each, by using Stimulator II (H Sachs). For each stimulation period, the right cardiac nerves were stimulated by rectangular impulses of 1–ms duration, 10 Hz and 35 mA. The interval between 2 stimulations was 30 min. To determine the output of NE, 4 samples were collected (each 15 s) of the venous effluent at 15, 30, 45 and 60 s after the electrical stimulation. NE was determined by a fluorometric method⁽⁵⁾. Freshly dissolved ramipril in Krebs-Henseleit solution was infused into the aorta cannula from 1 min after S_1 (10 $\mu\text{g}/\text{min}$) until the end of S_2 .

RESULTS

Effects of SNS on HR, FC, CF and NE release were summarized in Tab 1, and one typical experiment was illustrated as Fig 1. SNS of isolated rabbit hearts caused the rise in HR and FC. CF was initially decreased, but increased above the control levels after 30 s. SNS resulted in an increase of NE ranged from 2.8 ± 0.9 ng/ml (at 15 s during S_2) to 10.2 ± 2.7 ng/ml (at 45 s during S_1), $n=5$. After ramipril 10 $\mu\text{g}/(\text{ml}\cdot\text{min})$ was added to the perfusate for 30 min, the amount of NE released during SNS was significantly reduced ($n=5$).

Tab 1. Influence of ramipril on release of norepinephrine (NE) induced by cardiac sympathetic nerves stimulation (10 Hz, 35 mA, 1 ms) in isolated rabbit hearts. Two stimulation periods with intervals of 20–30 min were applied in each experiment. $n=5$, $\bar{x} \pm \text{SD}$. * $p > 0.05$, ** $p < 0.05$, *** $p < 0.01$ vs Stimulation₁.

Time of stimulation (s)	NE release (ng/ml)	
	Stimulation ₁	Stimulation ₂
Control		
15	3.7 ± 1.8	$2.8 \pm 0.9^*$
30	9.7 ± 2.1	$9.3 \pm 1.9^*$
45	10.2 ± 2.7	$9.3 \pm 1.9^*$
60	10.0 ± 2.3	$8.1 \pm 1.6^*$
Ramipril		
15	3.4 ± 1.3	$1.7 \pm 0.8^{**}$
30	8.4 ± 1.7	$3.4 \pm 1.5^{***}$
45	9.6 ± 2.0	$2.8 \pm 0.9^{***}$
60	8.2 ± 1.6	$2.1 \pm 0.9^{***}$

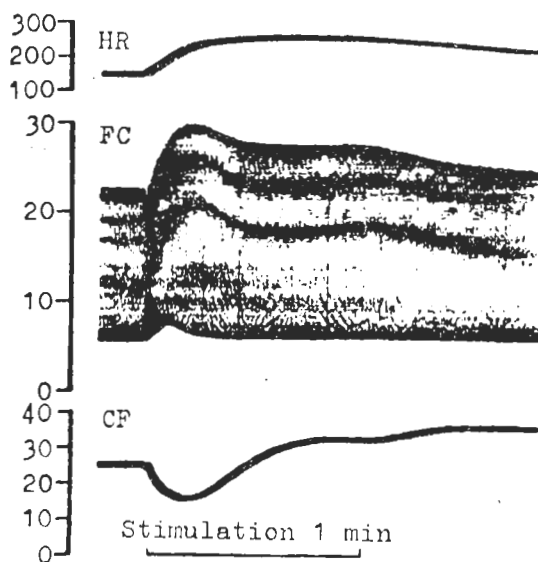


Fig 1. Effect of electrical sympathetic stimulation (10 Hz, 35 mA, 1 ms) of the cardiac nerve in isolated rabbit heart, HR=heart rate (bpm); FC=force of contraction (g); CF=coronary flow (ml/min).

DISCUSSION

The RAS other than the sympathetic nervous system in the regulation of blood pressure played an important role. ACEI strongly reduced the peripheral resistance

and lowered the blood pressure, but the precise mechanism of these effects were not elucidated⁽⁶⁾. Until recently, RAS inhibition was argued by Unger *et al* not to be the only mechanism by which they elicited antihypertensive effects, other factors might also be involved, e.g. by their interaction with the sympathetic nervous system through influences on angiotensin II synthesis⁽⁷⁾.

In the present study, it has been demonstrated that cardiac SNS resulted in an increase of NE, after the addition of ramipril to the perfusate, NE release was significantly reduced. The results suggest that the release of cardiac sympathetic neurotransmitter, NE, can be modified by the inhibition of this converting enzyme system.

Angiotensin II could facilitate the transmission of NE at the sympathetic ganglia and increase the release of NE from the adrenergic nerve terminals in the heart⁽⁸⁾. From our results it may be deduced that the inhibitory action of ACEI on the SNS-induced release of NE is related to the reduction of angiotensin I conversion in the heart, thus attenuates angiotensin II cardiac normal facilitating effect on the cardiac sympathetic neurotransmitter. This may contribute partly in the mechanism of the antihypertensive action of ACEI.

Thus, ramipril as an ACEI reduced NE release during cardiac sympathetic nerve stimulation. This effect may be involved in the angiotensin II-mediated facilitation of

adrenergic neurotransmission.

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雷米普利对离体兔心交感神经刺激期间去甲肾上腺素释放的影响

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提要 本研究旨在测定离体灌流兔心脏交感神经刺激期间去甲肾上腺素的释放及确定雷米普利(ramipril)对刺激所致去甲肾上腺素释放的抑制作用。交感神经刺激引起去甲肾上腺素释放量增加(2.8-10.2 ng/ml), ramipril 10 μg/(ml·min)加入灌流液 30 min 后, 去甲肾上腺素的释放量显著减少($p < 0.05-0.01$)。结果提

示: Ram 作为一种转换酶抑制剂, 可能干扰了肾上腺素能神经传递时血管紧张素介导的接通, 这一作用对解释转换酶抑制剂抗高血压作用机理是有帮助的。

关键词 雷米普利; 去甲肾上腺素; 交感神经系统; 激肽酶 II; 离体心脏