

INFLUENCE OF THE NOVEL NON-SULFHYDRYL CONVERTING ENZYME-INHIBITOR HOE-498 ON ISOLATED RAT HEARTS

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ABSTRACT In the isolated rat hearts, injection of Hoe-498 in cumulative doses of 0.1, 1, 3, 5 and 10 mg produced dose-dependent increases in coronary flow (CF) and force of contraction (FC).

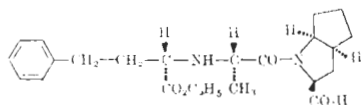
Angiotensin I (ANG I 100 ng) produced a decrease in CF and an increase in FC without an effect in heart rate (HR). Pretreatment of rats with intragastric Hoe-498 reduced the effects of ANG I on CF and FC.

Bradykinin (BK 10 ng) produced an increase in CF and a decrease in FC. Pretreatment of rats with Hoe-498 augmented the BK effect on CF and reversed the effect on FC.

Since Hoe-498 attenuates the cardiac effects of ANG I and potentiates BK actions, Hoe-498 is an effective inhibitor of converting enzyme (CE).

KEY WORDS isolated heart; Hoe-498; angiotensin I; bradykinin

The converting enzyme (CE), a peptidyl-dipeptide hydrolase, occurs in many tissues such as the heart⁽¹⁾. The heart and/or coronary vasculature can effect a significant conversion of the decapeptide ANG I to the vasoconstrictor octapeptide ANG II⁽²⁾, and the potent vasodilator nonapeptide BK is inactivated by the CE. Hoe-498, N-(1 (S)-carbethoxy-3-phenylpropyl)-(S)-alanyl-cis, endo-2-azabicyclo (3.3.0)-octan-3-(S)-carboxylic acid, is a novel orally active non-sulfhydryl CE-inhibitor.



To characterize its effect on the action of CE, we examined the influence of Hoe-498 on coronary flow (CF), heart rate (HR) and force of contraction (FC) as well as the effect of intragastric pretreatment on the actions of ANG I and BK in isolated perfused rat hearts.

MATERIALS AND METHODS

Sprague-Dawley ♀ rats (275±SD 6 g) were heparinized and Langendorff hearts were prepared under 85 cm H₂O. A perfusion period of 20-30 min was allowed for adaptation. The perfusate was a modified Krebs-Henseleit-bicarbonate solution containing (mM/L) NaCl 113.8, NaHCO₃ 22.0, KCl 4.7, KH₂PO₄ 1.2, MgSO₄·7H₂O 1.1, CaCl₂·2H₂O 2.5, Glucose 11, sodium pyruvate 2.0, gassed with 95% O₂ + 5% CO₂, pH 7.41, 38°C. Perfusate osmolality was kept at 270±4 mosmol/L by adjusting the amount of NaCl. Resting tension of heart = 2 g. Isometric contraction was recorded by a strain gauge transducer connected to a Heliscriptor He 19 (Hellige GmbH, Freiburg). CF was measured by a drop counter (7 drops/ml). Hearts were weighed when wet.

Hoe-498 was diluted in Krebs-Henseleit solution (0.5 mg/ml) and adjusted to pH 7.4. ANG I (Sigma Chem Co, St Louis) and BK (Paesel KG, Frankfurt am Main) were freshly prepared in Krebs-Henseleit solution and kept in an ice bath. The drugs were injected at a rate of 1 ml/min via aortic cannula into the inflow. For studying the effects of ANG I and BK, rats were pretreated by intragastric gavage with Hoe-498 1 mg/kg and Krebs-Henseleit solution 2 ml/kg as control 15 min, 1, 6, 24, 48 and 72 h before sacrifice.

Drug effects were pooled as $\bar{x} \pm SD$.

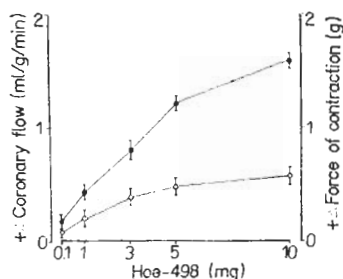


Fig 1. Influences of Hoe-498 on coronary flow (●) and force of contraction (○) in isolated rat hearts (n = 9)

Student's *t*-test was used for paired and unpaired comparisons.

RESULTS

Influence of Hoe-498 on isolated rat hearts (Fig 1) Nine hearts showed an initial CF of 8.1 ± 2.0 ml/g/min, FC of 3.48 ± 0.48 g, and HR of 288 ± 18 beats/min. Injections of Hoe-498 in cumulative doses of 0.1, 1, 3, 5 and 10 mg produced dose-dependent increases in CF 2-6-11-18-25%, and in FC 1-4-8-12-17%, without influencing HR.

Effects of ANG I and BK in isolated hearts pretreated with Hoe-498 (Tab 1)

Ten control hearts responded well to ANG I and BK. ANG I 100 ng caused CF to fall from 11.3 ± 3.2 to 8.6 ± 3.0 ml/g/min (-24%) and FC to rise from 3.4 ± 0.57 to 3.8 ± 0.57 g (+12%), without effect on HR. BK 10 ng caused CF to increase from 10.5 ± 3.0 to 11.5 ± 3.0 ml/g/min (+10%), and FC to decrease

from 4.1 ± 0.60 to 3.9 ± 0.57 g (-5%), without effect on HR.

After the pretreatment with Hoe-498, the effects of ANG I on CF and FC were significantly reduced, whereas the effects of BK on CF were potentiated and the negative inotropic effects of BK were reversed. Following the Hoe-498 pretreatment the ANG I and BK effects were significantly affected by CE-inhibition for 72 h.

DISCUSSION

The present findings about the effect of CE-inhibition in isolated perfused rat heart are indicative of a local conversion of ANG I to ANG II in the myocardium and coronary vasculature. It has been reported⁽²⁾ that the heart of rat contains as much as half the amount of CE found in the lung.

Our findings that ANG I increased the resistance of coronary vasculature, BK caused coronary vasodilation and the orally active CE-inhibitor Hoe-498 attenuated the cardiac effects of ANG I but potentiated BK actions are consistent with the observation of Gerlings and Gilmore⁽³⁾ and identical with the results in isolated guinea pig hearts^(4,5).

The coronary vasodilation induced by CE-inhibitors may be due either to inhibition of ANG I to ANG II conversion by the heart and/or its BK potentiating activity.

The result of an enhanced myocardial contraction in isolated rat hearts following ANG I

Tab 1. Changes of coronary flow and force of contraction induced by ANG I and BK in isolated rat hearts pretreated with Hoe-498. $\bar{x} \pm SD$. * $p > 0.05$, ** $p < 0.05$, *** $p < 0.01$

Time after Hoe-498	n	Angiotensin I(100 ng)				Bradykinin (10 ng)			
		Coronary flow (ml/g/min)		Force of contraction (g)		Coronary flow (ml/g/min)		Force of contraction (g)	
		-△	%	+△	%	+△	%	-△	%
—	10	2.76 ± 0.44	100	0.42 ± 0.13	100	1.00 ± 0.25	100	0.19 ± 0.06	100
15 min	10	$0.59 \pm 0.25^{***}$	21	$0.11 \pm 0.09^{***}$	26	$1.66 \pm 0.54^{***}$	166	0.16 ± 0.09	84
1 h	10	$0.60 \pm 0.19^{***}$	22	$0.13 \pm 0.09^{***}$	31	1.12 ± 0.44	112	$0.08 \pm 0.09^{***}$	42
6 h	9	$0.58 \pm 0.21^{***}$	21	$0.08 \pm 0.09^{***}$	19	$1.59 \pm 0.42^{***}$	159	$0.03 \pm 0.06^{***}$	16
24 h	10	$1.32 \pm 0.51^{***}$	48	$0.08 \pm 0.09^{***}$	19	1.08 ± 0.35	108	$0.04 \pm 0.06^{***}$	21
48 h	10	$1.62 \pm 0.54^{***}$	59	$0.24 \pm 0.09^{***}$	57	$1.50 \pm 0.79^*$	150	$0.08 \pm 0.09^{***}$	42
72 h	10	$2.26 \pm 0.66^*$	82	$0.26 \pm 0.09^{***}$	62	1.24 ± 0.63	125	$0.10 \pm 0.09^{**}$	53

injections can be explained by the fact that ANG II has a significant positive inotropic action on mammalian ventricular myocardium⁽⁶⁾. This effect may be caused either by an increased Ca^{++} entry into myocardial fibers⁽⁶⁾ or via ANG II receptors⁽⁷⁾, or may be the result of liberation of catecholamines from the cardiac stores⁽⁸⁾.

We conclude that Hoe-498 is a potent orally active converting enzyme inhibitor. Its effects of local inhibition of CE may have physiological importance for the regulations of coronary flow and force of myocardial contraction.

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