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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).

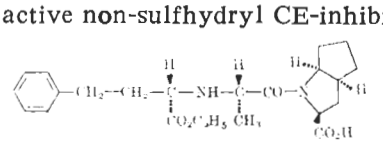
Angiotensin I (ANG I 100 ng) produced a decrease in CF and an increase in FC without an effect in heart rate (HR). Pretreatment of rats with intragastric Hoe-498 reduced the effects of ANG I on CF and FC.

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.



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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 H_2O. A perfusion period of 20-30 min was allowed for adaptation. The perfusate was a modified Krebs-Henseleit-bicarbonate solution containing (m*M*/L) NaCl 113.8, $NaHCO_3$ 22.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.1, $CaCl_2 \cdot 2H_2O$ 2.5, Glucose 11, sodium pyruvate 2.0, gassed with 95%O_2 + 5%CO_2, pH 7.41, 38℃. 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 $\bar{x}$±SD.

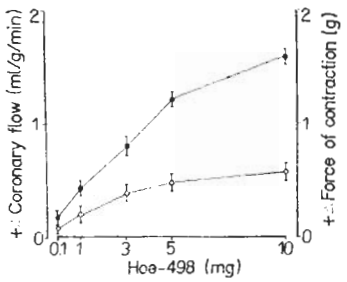

Fig 1. Influences of Hoe-498 on coronary flow (●) and force of contraction (○) in isolated rat hearts (n = 9)

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) Coronary flow (ml/g/min) −△	%	Force of contraction (g) +∧	%	Bradykinin (10 ng) 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±0.25***	21	0.11±0.09***	26	1.66±0.54***	166	0.16±0.09	84
1 h	10	0.60±0.19***	22	0.13±0.09***	31	1.12±0.44	112	0.08±0.09***	42
6 h	9	0.58±0.21***	21	0.08±0.09***	19	1.59±0.42***	159	0.03±0.06***	16
24 h	10	1.32±0.51***	48	0.08±0.09***	19	1.08±0.35	108	0.04±0.06***	21
48 h	10	1.62±0.54***	59	0.24±0.09***	57	1.50±0.79*	150	0.08±0.09***	42
72 h	10	2.26±0.66*	82	0.26±0.09***	62	1.24±0.63	125	0.10±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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REFERENCES

1 Cushman DW, Cheung HS(张洪声). *Biochim Biophs Acta* 1971; 250 : 261
2 Huggins CG, Thampi NS. *Life Sci* 1968; 7 : 633
3 Gerlings ED, Gilmore JP. *Basic Res Cardiol* 1973; 69 : 222
4 Linz W, Xiang JZ, Schölkens BA, Unger T. to be published
5 Xiang JZ, Schölkens BA, Linz W, Unger T. to be published
6 Koch-Weser J. *Circ Res* 1965; 16 : 230
7 Kobayashi M, Furukawa Y, Chiba S. *Eur J Pharmacol* 1978; 50 : 17
8 Beaulines A. *Biochem Pharmacol* 1983; 12(suppl) :181