Influence of the Novel Non-Sulphydryl Converting Enzyme-Inhibitor Hoe 488 on Isolated Rat Hearts

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ABSTRACT In the isolated rat hearts, injection of Hoe-488 in cumulative doses of 0.1, 1, 3, 5 and 10 ng produced dose-dependent increases in coronary flow (CF) and force of contract (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-488 reduced the effect 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-488 augmented the BK effect on CF and reversed the effect on FC.

Since Hoe-488 attenuates the cardiac effects of ANG I and potentiates BK actions, Hoe-488 is an effective inhibitor of converting enzyme (CE).

Key Words isolated heart; Hoe-488; 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 affect a significant conversion of the decapeptide ANG I to the vasconstrictor octapeptide ANG II\(^2\), and the potent vasodilator nonapeptide BK is inactive by the CE.

Hoe-488, N\((\text{S-carboxy-3-phenylpropyl})-\text{(S)-alanine-endo}-3\)-azacyclo(3.3.0)-oct-1-ene-\((3,3\)-dihydroxy-3\)-carboxylic acid, is a novel orally active non-sulphydryl CE-inhibitor.

To characterize its effect on the action of CE, we examined the influence of Hoe-488 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±5SD g) were heparinized and left endotracheally intubated. They were prepared under 85 cm H\(_2\)O. A perfusion period of 20-30 min was allowed for adaptation. The perfusate was a modified Krebs-Henseleit-bicarbonate solution containing (nM/L) NaCl 113.8, NaHCO\(_3\) 22.0, KCl 4.7, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 7H\(_2\)O 1.1, CaCl\(_2\) 2H\(_2\)O 2.5, Glucose 11, sodium pyruvate 3.0, passed with 35/5 CO\(_2\)/O\(_2\), pH 7.4, 37°C. Perfusion osmolality was kept at 270±2 mmosm/L by adjusting the amount of NaCl. Resting tension of heart = 5 g. Isometric contraction was recorded by a strain gauge transducer connected to a Hellertror He 79 (Hellige GmbH, Freiburg). CF was measured by a drop counter (7 drops/ml). Hearts were weighed when wet.

Hoe-488 was diluted in Krebs-Henseleit solution (0.5 mg/ml) and adjusted to pH 7.4. ANG I (Sigmas Chem Co, St Louis) and BK (Pascet 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-488 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 \(\bar{x}±SD\).
**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. PC 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, and 10 mg produced dose-dependent increases in CF 2.6-117% and in PC 1-18% 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 PC to rise from 3.4±0.7 to 3.8±0.5 g (+12%), without effect on HR. BK 10 ng caused CF to decrease from 10.5±3.0 to 11.5±3.0 ml/g/min (+10%) and PC to decrease from 4.1±0.6 to 3.9±0.5 g (-5%), without effect on HR.

The effects of ANG I on CF and PC 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 treatment the effects of ANG I and BK 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 that the hearts of rat contain 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 and identical with the results in isolated guinea pig hearts.

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 results of an enhanced myocardial contraction in isolated rat hearts following ANG I pretreatment with Hoe-498 are presented in Table 1.
injections can be explained by the fact that ANG II has a significant positive inotropic action on mammalian ventricular myocardium\(^\text{19}\). This effect may be caused either by an increased Ca\(^{\text{2+}}\) entry into myocardi al fibers\(^\text{16}\) or via ANG II receptors\(^\text{17}\). It may be the result of liberation of catecholamines from the cardiac stores\(^\text{18}\).

We conclude that Hou-408 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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