



Inhibition of neutral endopeptidase by thiorphan does not modify coronary vascular responses to angiotensin I, angiotensin II and bradykinin in the isolated guinea pig heart

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Abstract:

Both angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP) are involved in the regulation of renin-angiotensin and kallikrein-kinin systems. The aim of the present study was to assess the role of NEP and ACE in the regulation of vascular responses to angiotensin I (Ang I), angiotensin II (Ang II) and bradykinin (Bk) in the coronary circulation. For this purpose we used typical inhibitors of ACE and NEP, perindoprilate (1 μM) and thiorphan (1 μM and 10 μM), respectively, and analyzed their effects on the coronary vasoconstrictor responses to Ang I and Ang II and coronary vasodilator responses to Bk in the isolated guinea pig heart. Perindoprilate abolished coronary vasoconstriction induced by Ang I and potentiated coronary vasodilation evoked by Bk. Thiorphan at a concentration of 1 μM slightly reduced response to Ang I without a significant effect on the responses to Ang II and Bk. However, thiorphan at a concentration of 10 μM abolished coronary vasoconstrictor response to Ang I and enhanced Bk-induced vasodilation. Importantly, in the presence of perindoprilate, addition of thiorphan (10 μM) did not modify further either responses to Ang I, Ang II or to Bk. In conclusion, vascular responses induced by Ang I, Ang II and Bk in the isolated guinea pig heart are regulated by ACE but not by NEP. Moreover, thiorphan is not a perfect tool to assess functional role of NEP as it displays ACE inhibitory activity.

Key words:

angiotensin, angiotensin-converting enzyme, neutral endopeptidase, coronary vessels, guinea pig heart

Abbreviations: ACE – Ang-converting enzyme, ACE-I – Ang-converting enzyme inhibitor, Ang – angiotensin, ANP – atrial natriuretic peptide, Bk – bradykinin, BNP – brain natriuretic peptide, CNP – natriuretic peptide type C, CF – coronary flow, LVP – left-ventricular pressure, NEP – neutral endopeptidase, NEP-I – neutral endopeptidase inhibitor

Introduction

Angiotensin converting enzyme inhibitors (ACE-I) are commonly used in the treatment of arterial hypertension and congestive heart failure. Many clinical tri-

als confirmed their beneficial effects: reduced risk of death, myocardial infarction and stroke [6, 34]. ACE not only converts angiotensin I (Ang I) to a strong vasoconstrictor angiotensin II (Ang II), but it is also responsible for the degradation of a strong vasodilator bradykinin (Bk) as well des-Arg⁹-Bk to inactive products in endothelium, cardiac and vascular tissues [6].

On the other hand, neutral endopeptidase, or neprilysin (NEP) is primarily responsible for degradation of natriuretic peptides, such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and natriuretic peptide type C (CNP) [5, 6], known for their hypotensive, antiproliferative, natriuretic and vasculoprotective properties [3, 5, 6, 28]. NEP is also an important enzyme of the renin-angiotensin system as it converts Ang I to Ang-(1–7) and Ang II to Ang-(1–4) [10]. Furthermore, it was also shown that NEP was one of the key enzymes responsible for the degradation of Bk in the kidney [12, 27] and in endothelial cells [13, 20]. Interestingly, inhibitors of NEP not only slowed degradation of Bk, but also resensitized B₂ receptors after their desensitization by Bk [8]. Bk-dependent cardioprotective action of NEP inhibition has been recently demonstrated in the rabbit model of myocardial infarction [23].

It was claimed that dual inhibition of ACE and NEP might have therapeutic advantage over the inhibition of ACE alone in heart failure [39], in diabetes mellitus [32] or in hypertension [26]. On the other hand, no advantage of dual ACE and NEP inhibition over the inhibition of ACE alone was found in the treatment of endothelial dysfunction and experimental atherosclerosis [37]. Similarly in clinical trials, in two studies, IMPRESS and OVERTURE advantage of omapatrilat over an ACE inhibitor was demonstrated in patients with congestive heart failure in terms of risk of death or hospitalization [25, 31]. On the other hand, even though omapatrilat was found to be superior to enalapril in hypertensive patients in OCTAVE trial, there was approximately 3-fold higher incidence of the dangerous adverse effect of the drug (angioedema) in the group of patients treated with omapatrilat [16].

Although the major mechanism of the beneficial effects of NEP inhibition seems to be related to the inhibition of degradation of natriuretic peptides, the involvement of Bk is also possible. On the other hand, the decreased formation of Ang-(1–7) from Ang I and Ang II as well as the increased generation of Ang II or endothelin-1 [3, 11, 14] may blunt positive effect of NEP inhibition and enhance vascular tone.

The aim of this study was, therefore, to assess the role of NEP and ACE in the regulation of vascular responses to Ang I, Ang II and Bk in the coronary circulation of the isolated guinea pig heart. For that purpose, we assessed coronary responses to Ang I, Ang II and Bk, in the absence and in the presence of ACE and/or NEP inhibitors.

Materials and Methods

Perfusion of the isolated guinea pig heart

The details of the method were described elsewhere [17, 18]. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and the experimental procedures used in the present study were approved by the local Animal Research Committee. Briefly, guinea pigs of both sexes and body weight of 255–860 g were anesthetized with thiopental (25–100 mg/kg). Their hearts were isolated, washed in ice-cold saline, and mounted in Langendorff apparatus of Hugo Sachs Electronics (HSE). Guinea pig hearts were perfused retrogradely through aorta under a constant perfusion pressure of 60 mmHg with Krebs-Henseleit buffer of the following composition (mM): NaCl 118.06, KCl 4.69, CaCl₂ 2.52, MgSO₄ 1.16, NaHCO₃ 25.00, KH₂PO₄ 1.19, glucose 10.00, sodium pyruvate 2.00, equilibrated with 95% O₂ + 5% CO₂ at 37°C in an oxygenator with rotating disc (HSE). The hearts were paced with 273 impulses per min through two platinum electrodes placed in the right atrium. Coronary flow (CF) was monitored by Ultrasonic flowmeter (HSE) and finally analyzed using the specially designed software (PSCF – IGEL, Poland).

Protocol of experiments

The isolated heart of the guinea pig was equilibrated at a perfusion pressure of 50 mmHg for about 10 min, after which the pressure was adjusted to 60 mmHg. At that pressure the heart was further equilibrated for 10–15 min before the beginning of the experiment. The heart was used for the experiment only if the following criteria were fulfilled: (i) basal CF was 7–20 ml/min, (ii) the increase in CF to bolus injection of

300 pmoles of acetylcholine was > 2 ml/min, (iii) the increase in CF induced by 15 s of coronary occlusion amounted to at least 5 ml/min, (iv) Ang I or Ang II (each at 100 pmoles) decreased CF by at least 2 ml/min.

The involvement of ACE and NEP in vascular responses to Ang I, Ang II and Bk was assessed by pre-treatment with perindoprilate (1 μ M) or thiorphan (1 or 10 μ M), respectively. In some experiments, another NEP inhibitor, phosphoramidon (10 μ M) was used instead of thiorphan. In each experiment, bolus injections of Ang I (10 pmoles), Ang II (100 pmoles) and Bk (1 or 3 pmoles) were carried out in the absence and then in the presence of perindoprilate, thiorphan or phosphoramidon. Also effects of NEP-I on responses induced by Ang I, Ang II and Bk were assessed in hearts pretreated with ACE-I from the beginning of the experiment. In each experimental group, *n* denotes number of isolated heart experiments.

Ang I, Ang II, Bk were dissolved in distilled water and were applied as bolus injections in the volume of 10–30 μ l. Perindoprilate, thiorphan and phosphoramidon were dissolved in saline and were added to Krebs-Henseleit buffer and perfused through the heart for at least 15 min before eliciting responses to Ang I, Ang II or Bk.

Ang I, Ang II, Bk, thiorphan and phosphoramidon were purchased from Sigma Chemicals International. Perindoprilate was kindly donated by Servier.

The duration of an experiment never exceeded three hours, and for this period the quality of preparation of the isolated guinea pig heart stayed mostly unchanged.

Statistical analysis

All values are expressed as the mean \pm SEM of change in CF (ml/min). Statistical significance of changes in the same heart (i.e. before and after adding inhibitor) was assessed by paired Student's *t*-test, * denotes $p < 0.05$, ** $p < 0.01$ and *** $p < 0.005$.

Results

Coronary flow responses to Ang I, Ang II and Bk in the isolated guinea pig heart

The mean basal coronary flow (CF) in 41 isolated hearts of guinea pig used in experiments was $12.40 \pm$

0.51 ml/min. Ang I or Ang II (each at a dose of 100 pmoles) decreased CF by 4.01 ± 0.29 ml/min ($n = 25$) and 4.28 ± 0.29 ml/min ($n = 25$), respectively, while Bk at a dose of 1 and 3 pmoles increased CF by 4.38 ± 0.61 ml/min ($n = 36$) and 5.91 ± 1.06 ml/min ($n = 12$), respectively.

Effects of the inhibition of ACE with perindoprilate

Infusion of perindoprilate (1 μ M) decreased basal CF from 13.36 ± 1.01 ml/min to 10.58 ± 1.02 ml/min ($79.90 \pm 5.32\%$, $n = 9$, $p = 0.006^{**}$). In the presence of perindoprilate, response to Ang I was abolished (from -4.74 ± 0.53 ml/min to 0.00 ± 0.07 ml/min, $n = 8$, $p = 0.000024^{***}$) whereas response to Ang II was not significantly changed (-4.82 ± 0.49 ml/min and -4.13 ± 0.62 ml/min $n = 8$, $p = 0.144$, before and after addition of perindoprilate, respectively). On the other hand, response to Bk was potentiated by perindoprilate (for 1 pmoles of Bk 4.55 ± 0.82 ml/min vs. 8.49 ± 1.14 ml/min, $n = 4$, $p = 0.029^*$, before and after perindoprilate, respectively; Fig. 1A, B).

Effects of the inhibition of NEP with thiorphan

Infusion of thiorphan alone at a concentration of 1 μ M did not change significantly basal CF (12.41 ± 1.13 ml/min before thiorphan and 11.52 ± 1.31 ml/min after thiorphan, $n = 9$, $p = 0.441$), whereas thiorphan at a concentration of 10 μ M decreased basal CF from 12.34 ± 1.35 ml/min to 8.90 ± 1.13 ml/min ($n = 10$, $p = 0.032^*$). In the presence of 1 μ M thiorphan the vasoconstrictor response induced by Ang I (100 pmoles) was inhibited (-3.84 ± 0.47 ml/min vs. -2.34 ± 0.44 ml/min, before and after thiorphan, respectively, $n = 9$, $p = 0.002^{***}$), whereas responses to Ang II and to Bk were unchanged (Fig. 2A). Thiorphan given at a concentration of 10 μ M almost completely inhibited coronary vasoconstriction induced by Ang I (-3.78 ± 0.54 ml/min vs. -0.13 ± 0.07 ml/min, before and after thiorphan, respectively $n = 7$, $p = 0.00049^{***}$) and augmented coronary vasodilation induced by Bk (for 1 pmole of Bk 3.12 ± 0.49 ml/min vs. 4.20 ± 0.61 ml/min, $n = 6$, $p = 0.335$, for 3 pmoles of Bk, 6.57 ± 1.57 ml/min vs. 9.99 ± 1.55 ml/min $n = 6$, $p = 0.017^*$, before and after thiorphan, respectively, Fig. 2B).

Addition of thiorphan (10 μ M) to hearts pretreated with perindoprilate (1 μ M) did not significantly

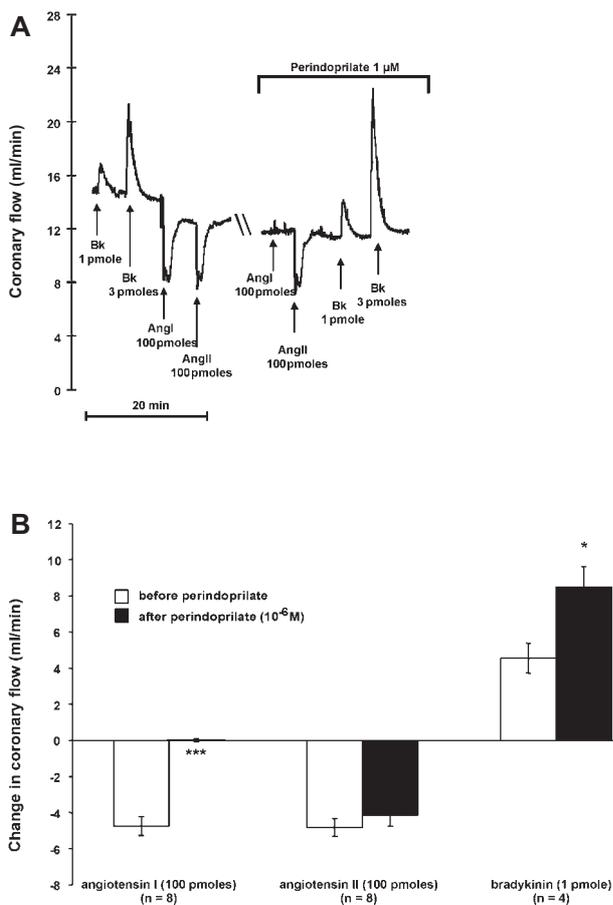


Fig. 1. Effects of perindoprilate (1 μ M) on the coronary vascular responses induced by Ang I, Ang II and Bk. **(A)** A representative tracing of the experiment **(B)** Summarized data from $n = 4$ –8 experiments. The data are expressed as the mean \pm SEM, * and *** denotes statistically significant difference as compared to the response before perindoprilate ($p < 0.05$ and $p < 0.005$, respectively)

change basal CF (11.60 ± 0.91 ml/min vs. 12.02 ± 1.02 ml/min, $n = 9$, $p = 0.413$, before and after thiorphan, respectively). Thiorphan did not change response to Ang I (0.67 ± 0.78 ml/min vs. 0.15 ± 0.54 ml/min, with perindoprilate alone and perindoprilate + thiorphan, respectively, $n = 5$, $p = 0.138$), to Ang II (-4.53 ± 0.98 ml/min vs. -5.31 ± 1.58 ml/min, before and after thiorphan, respectively, $n = 4$, $p = 0.289$) as well as to Bk (7.95 ± 1.45 ml/min vs. 7.95 ± 1.51 ml/min $n = 7$, $p = 0.954$ for 1 pmole of Bk, before and after thiorphan, respectively).

Similarly to thiorphan, phosphoramidon (10 μ M) did not modify responses to Ang I, Ang II and Bk in the presence of ACE inhibition by perindoprilate (1 μ M, data not shown).

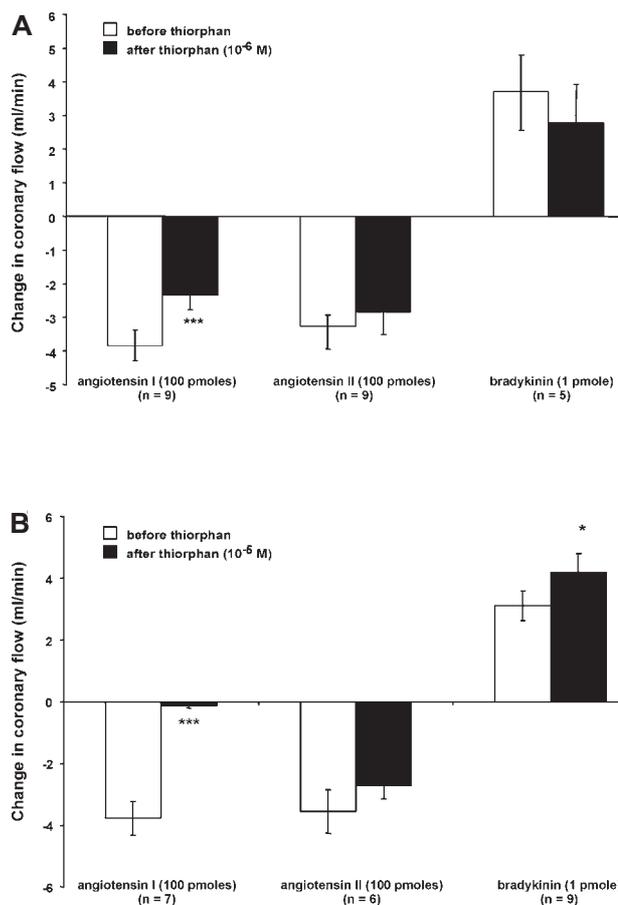


Fig. 2. Effects of thiorphan at the concentrations of 1 μ M **(A)** and 10 μ M **(B)** on the coronary vascular responses induced by Ang I, Ang II and Bk. The data are expressed as the mean \pm SEM, * and *** denotes statistically significant difference as compared to the response before thiorphan ($p < 0.05$ and $p < 0.005$, respectively)

Discussion

In the present work, we confirmed the key role of ACE in the conversion of Ang I to Ang II and in the inactivation of Bk and excluded the important role of NEP in the regulation of coronary vascular responses to Ang I, Ang II and Bk in the isolated guinea pig heart.

The major role of ACE in the conversion of Ang I and Bk in the coronary circulation was demonstrated previously in numerous studies both in the isolated heart model [1, 33, 38] and in the isolated coronary arteries [15, 36]. However, the contribution of NEP to

the regulation of vascular tone by Ang and Bk has been less intensively studied.

In the present work, we used thiorphan that has been used previously as a NEP inhibitor [7, 19, 21]. Indeed, it was reported that thiorphan augmented the coronary vasodilator response to CNP in the isolated porcine coronary artery [21]. In our experiments in the presence of thiorphan (1 μ M) coronary vasodilator response to CNP was also augmented (data now shown).

We found that thiorphan at a higher concentration (10 μ M) slightly amplified the coronary vasodilatation induced by Bk as also reported previously [4, 7, 19]. Obviously, Bk-potentating effect of thiorphan may be due to ACE inhibition afforded by higher concentration of thiorphan [22]. Indeed, thiorphan at a lower concentration of 1 μ M, failed to augment Bk-induced coronary vasodilatation in the isolated guinea pig heart. Moreover, in the experiments in which ACE was inhibited by perindoprilate from the beginning of the experiment, thiorphan (10 μ M) did not amplify responses to Bk. These results strongly suggest that the effect of thiorphan on Bk response was indeed due to the inhibition of ACE rather than the inhibition of NEP. Lack of the effect of phosphoramidon, another NEP inhibitor without ACE inhibitory activity [8, 21, 35] on response to Bk seems to support our notion that NEP does not contribute to Bk metabolism in the coronary circulation of the isolated guinea pig heart and that enhancement of the Bk-induced vasodilatation afforded by higher concentration of thiorphan was mediated by ACE inhibition.

ACE inhibition by thiorphan was also evidenced by the inhibitory effect of thiorphan on vasoconstrictor response to Ang I. Indeed, at a concentration of 10^{-6} M thiorphan slightly inhibited and at a concentration of 10^{-5} M abolished response to Ang I. Obviously, this effect cannot be mediated *via* NEP inhibition, since this enzyme may only convert Ang I to vasodilator Ang-(1-7) [10, 40]. Furthermore, our results seem also to exclude the major role of NEP in the formation of Ang-(1-7) from Ang I in this preparation as it was the case in the isolated rat lung [2].

It was also reported that NEP inhibition enhanced vasoconstrictor response to Ang II in humans [29]. This was due to participation of NEP in Ang II metabolism to inactive Ang-(1-4) [10]. Again, we did not observe augmentation of the coronary vasoconstrictor response to Ang II by thiorphan in the isolated guinea pig heart. Accordingly, the contribution of NEP to the further metabolism of Ang II in the coro-

nary vessels of the isolated guinea pig heart does not seem to bear important functional consequences.

It is worth to add that our results do not exclude a possibility that in some pathologic conditions the role of NEP in the coronary circulation may appear more important, as it was observed in the experimental model of myocardial infarction in rats [9] or after the long-term inhibition of ACE [24]. Also, in patients with congestive heart failure treated by ACE inhibitors thiorphan enhanced Bk-induced increase in forearm blood flow [7]. NEP may be also of significance in the regulation of vascular NO production in microvessels [41]. Furthermore, NEP-dependent transformation of Ang I to Ang-(1-7) is increased in patients with congestive heart failure [42].

In conclusion, our results do not support the significant role of NEP in the regulation of the vascular responses to Ang I, Ang II and Bk in the healthy guinea pig heart. ACE plays a dominant role in the conversion of Ang I to Ang II and in the degradation of Bk in this preparation. Our results also showed that thiorphan was not a perfect tool for investigation of the biological role of NEP as it displays ACE inhibitory properties. The use of other more selective NEP inhibitors is recommended [30].

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