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Reciprocal Regulation of Angiotensin Converting Enzyme and Neutral Endopeptidase in Rats with Experimental Hypertension

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Summary

Angiotensin I is a substrate for both the angiotensin converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP). The present study examined the hypothesis that a high ACE expression is associated with a low NEP activity. Male Sprague-Dawley rats had two-kidney, one-clip (2K1C), N^G-nitro-L-arginine methyl ester (L-NAME) or deoxycorticosterone acetate (DOCA)-salt hypertension, which were either angiotensin-dependent or -independent. The expression of ACE and NEP mRNA was determined in the thoracic aorta by a reverse transcription-polymerase chain reaction. The catalytic activity of NEP was measured by fluorometry. The expression of ACE was increased in 2K1C and L-NAME hypertension, and decreased in DOCA-salt hypertension. Conversely, the expression of NEP was decreased in 2K1C and L-NAME hypertension, and increased in DOCA-salt hypertension. The catalytic activity of NEP was altered similarly as its expression. These results suggest that ACE expression is inversely related to vascular NEP activity in certain forms of hypertension.

Key words
Angiotensin-converting enzyme • Neutral endopeptidase

Introduction

Angiotensin I is a substrate for both the angiotensin converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP) (Welches *et al*. 1993). ACE plays an important role in regulation of the overall activity of the renin-angiotensin system (RAS) by hydrolyzing angiotensin I to angiotensin II. On the other hand, the action of NEP on angiotensin I generates angiotensin-(1-7) heptapeptide with vasodilator activity. The physiological effects of angiotensin II and angiotensin-(1-7) are opposite to each other: the former elevates and the latter lowers the blood pressure (Ferrario *et al.* 1997). An interaction between NEP and ACE may thus modulate the overall activity of RAS.

It has been shown that genetically determined high ACE expression is inversely related to tissue NEP activity, which can result in low tissue levels of angiotensin-(1-7) (Oliveri *et al.* 2001). However, it has not been explored whether NEP activity is modulated by an altered ACE expression in which the latter has been secondarily determined. The present study was aimed to ascertain a reciprocal regulation between ACE expression (secondarily determined) and NEP activity. The tissue NEP activity was measured in the vasculature of rats the RAS activity of which was either activated or inactivated.

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Methods

Animals

Male Sprague-Dawley rats, weighing 200-250 g, were used. They were given a standard rat chow with salt and water *ad libitum*. In order to study animals with activated or inactivated RAS, the experiments were performed in rats with two-kidney, one-clip (2K1C), N^G-nitro-L-arginine methyl ester (L-NAME), or deoxycorticosterone acetate (DOCA)-salt hypertension. They were used 4 weeks after inducing the hypertension. All experiments were performed following the recommendations of the Guide for the Care and Use of Laboratory Animals (NIH publication No. 93-23, revised 1985).

Determination of ACE and NEP expression

The expression of ACE and NEP was determined in the thoracic aorta by reverse transcription-polymerase chain reaction (RT-PCR). PCR primers for ACE amplification of 389 bp fragments were sense 5'-GCCTCCCCAACAAGACTGCCA-3' and antisense 3'-CCACATGTCTCCCCAGCAGATG-5'. Those for NEP (EC 3.4.24.11) amplification of 222 bp fragments were sense 5'-TTCTGTGCTCGTCTTGCTCC-3' and antisense 3'-CTGTATCGGGAACTGGTCTC-5'.

NEP activity

For the measurement of tissue NEP activity, the thoracic aorta was quickly removed, washed extensively with cold saline solution to remove all blood, and homogenized in 10 volumes of cold 50 mmol/l Tris-HCl buffer (pH 7.4). The homogenate was centrifuged for 5 min at 1,000 × *g* at 4 °C. The pellet was discarded and the supernatant centrifuged at 60,000 × *g* for 60 min at 4 °C. The resulting pellet was superficially washed 3 times with a cold buffer and resuspended in 50 mmol/l Tris-HCl buffer (pH 7.4) and used as the enzymatic source. Protein was measured by the method of Bradford (1976).

NEP activity was fluorometrically estimated as described previously (Florentin *et al.* 1984). Briefly, 100 μg protein were incubated for 30 min at 37 °C with 50 μmol/l dansyl-D-Ala-Gly-p-nitro-Phe-Gly (DAGPNG, Sigma), 200 μmol/l enalapril (Laboratorio Saval), and 50 mmol/l Tris-HCl buffer (pH 7.4) in the presence or absence of 20 nmol/l thiorphan (Sigma). Enzymatic reactions were stopped by boiling at 95 °C for 5 min. The samples were then diluted with Tris-HCl buffer and centrifuged at 5,000 × *g* for 30 min. The fluorescence of the supernatant was measured with a fluorescence spectrofluorometer at 562 nm (λex 342 nm). The calibration curve was prepared by adding increasing concentrations of dansyl-D-Ala-Gly (DAG, Sigma) (1 to 10 μmol/l) and decreasing concentrations of DAGPNG (50 to 40 μmol/l). NEP activity was expressed as U/mg protein.

Statistical analysis

Results are shown as mean ± S.E.M. Comparisons between the groups were performed with Student's *t* test for independent measurements or with the Mann Whitney test. $P<0.05$ value was considered as statistically significant.

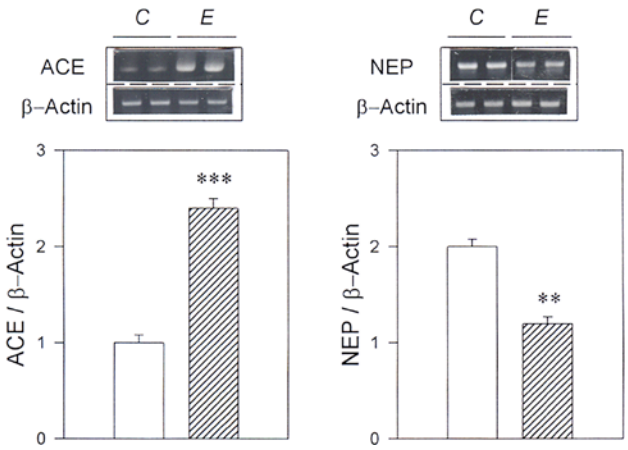

Fig. 1. Expression of angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) in 2K1C hypertension. Representative ethidium bromide-stained gels and densitometric data are shown. Each column represents mean ± S.E.M. of 6 rats (open, control; hatched, experimental). *p<0.05, **p<0.01; compared with control.

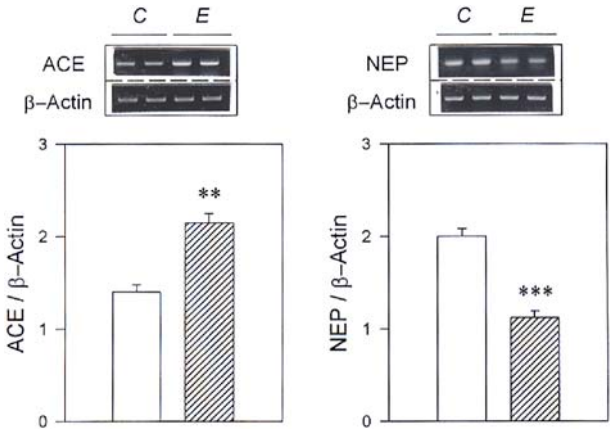

Fig. 2. Expression of ACE and NEP in L-NAME hypertension. Representative ethidium bromide-stained gels and densitometric data are shown. Each column represents mean ± S.E.M. of 6 rats (open, control; hatched, experimental). *p<0.05, **p<0.01; compared with control.

Results

The aortic expression of ACE mRNA was increased in 2K1C and L-NAME hypertension, and decreased in DOCA-salt hypertension (Figs. 1-3). On the contrary, the aortic mRNA expression of NEP was decreased in 2K1C and L-NAME hypertension, and increased in DOCA-salt hypertension (Figs. 1-3). The aortic tissue activity of NEP was altered in a similar manner as NEP expression (Fig. 4).

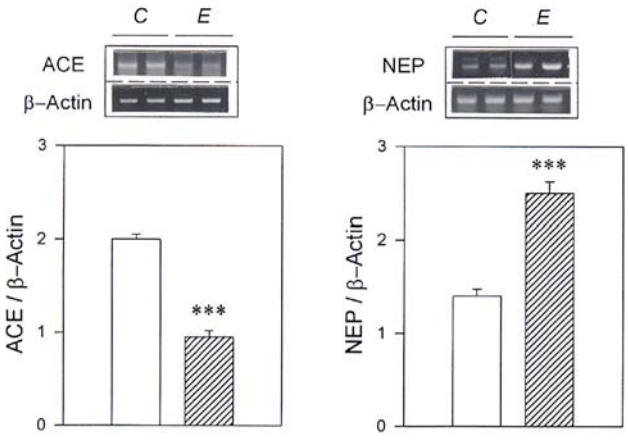

Fig. 3. Expression of ACE and NEP in DOCA-salt hypertension. Representative ethidium bromide-stained gels and densitometric data are shown. Each column represents mean ± S.E.M. of 6 rats (open, control; hatched, experimental). *p<0.05, **p<0.01; compared with control.

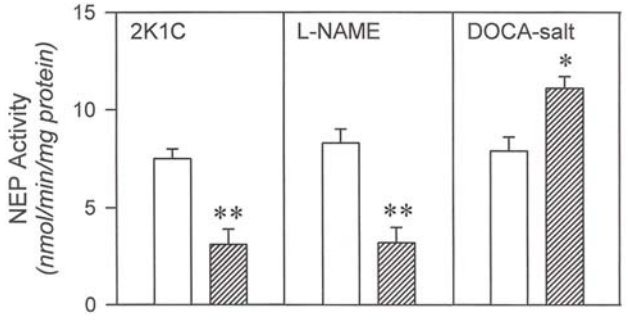

Fig. 4. Activity of NEP in 2K1C, L-NAME, and DOCA-salt hypertension. Each column represents mean ± S.E.M. of 6 rats (open, control; hatched, experimental). *p<0.05, **p<0.01; compared with control.

Discussion

The present study investigated the expression of ACE and NEP in the thoracic aorta of rats with different forms of hypertension in which RAS was either activated or inactivated. In 2K1C and L-NAME hypertension, the increased expression of ACE was associated with a decreased NEP expression. On the contrary, in DOCA-salt hypertension, the expression of ACE was decreased along with an increased NEP expression. It has been shown in homozygous rats that NEP activity is inversely related with ACE activity (genetically determined) (Oliveri *et al.* 2001). Although the expression of ACE was differently determined, genetically vs. secondarily, both studies confirm the hypothesis that ACE and NEP are reciprocally regulated.

Because NEP is a potent inactivator of vasoactive peptides and also generates angiotensin-(1-7), the alterations of its expression and activity may influence local vasomotor responses (Skidgel *et al.* 1984, Erdös and Skidgel 1989, Yamoto *et al.* 1992). The vascular action of angiotensin-(1-7) is partly related to the release of either nitric oxide or vasodilator prostaglandins (Benter *et al.* 1993, Osei *et al.* 1993, Porsti *et al.* 1994). Therefore, a reduced NEP activity in the presence of high ACE expression may result in a prolonged increase of angiotensin II levels as well as the decreased generation of angiotensin-(1-7), contributing to the elevation of vascular tone and blood pressure.

It has been shown that NEP is especially active when ACE is inhibited (Graf *et al.* 1993). Furthermore, Drummer *et al.* (1990) have suggested that chronic treatment with ACE inhibitors affects NEP activity, causing substantial changes in angiotensin metabolism. Indeed, the vasodilator effect caused by combined inhibition of ACE and type-1 angiotensin II receptors has been shown to be mediated by an important contribution of angiotensin-(1-7) (Iyer *et al.* 1998). The effects of ACE inhibitors in reducing the blood pressure could in part depend on the interaction of ACE expression with NEP activity. The enhanced NEP activity associated with a decreased ACE expression results in accumulation of vasodilator angiotensin-(1-7) as well as in a decreased formation of vasoconstrictor angiotensin II, contributing to the counteraction of the high blood pressure in certain forms of hypertension.

It may be of interest to note that separate inhibition of either ACE or NEP has been shown to affect both enzymes, with varying potency in different tissues (Helin *et al.* 1994). It is uncertain whether ACE activity has a direct effect at the level of NEP expression. Mechanisms underlying the modulating effect of ACE expression on NEP levels and activity remain to be clarified. Further studies using NEP or ACE inhibitors in

different models of hypertension may determine whether there is a differential pharmacological and biological response to both inhibitors.

In summary, the present study has shown a reciprocal regulation of ACE and NEP gene expression in the vasculature: a high ACE expression is associated with low NEP activity (and *vice versa*).

Acknowledgements

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