Neutral endopeptidase inhibition potentiates the natriuretic actions of adrenomedullin

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Lisy, Ondrej, Michihisa J ougasaki, J ohn A. Schirger, Horng H. Chen, Paul T. Barclay, and John C. Burnett, Jr. Neutral endopeptidase inhibition potentiates the natriuretic actions of adrenomedullin. Am. J. Physiol. 275 (Renal Physiol. 44): F410–F414, 1998.—Adrenomedullin (ADM) is a potent renal vasodilating and natriuretic peptide possessing a six amino acid disulfide ring. Neutral endopeptidase 24.11 (NEP) is localized in greatest abundance in the kidney and cleaves endogenous peptides like atrial natriuretic peptide, which also possesses a disulfide ring. We hypothesized that NEP inhibition potentiates the natriuretic actions of exogenous ADM in anesthetized dogs (n = 6). We therefore investigated renal function in which one kidney received intrarenal infusion of ADM (1 ng·kg⁻¹·min⁻¹) while the contralateral kidney served as control before and during the systemic infusion of a NEP inhibitor (Candoxatrilat, 8 µg·kg⁻¹·min⁻¹; Pfizer). In response to ADM, glomerular filtration rate (GFR) in the ADM kidney did not change, whereas renal blood flow, urine flow (UV), and urinary sodium excretion (UNaV) increased from baseline. Proximal and distal fractional reabsorption of sodium decreased in the ADM-infused kidney. In response to systemic NEP inhibition, UNaV and UV increased further in the ADM kidney. Indeed, ΔUNaV and ΔUV were markedly greater in the ADM kidney compared with the control kidney. Plasma ADM was unchanged during ADM infusion but increased during NEP inhibition. In conclusion, the present investigation is the first to demonstrate that NEP inhibition potentiates the natriuretic and diuretic responses to intrarenal ADM. This potentiation occurs secondary to a decrease in tubular sodium reabsorption. Lastly, the increase in plasma ADM during systemic NEP inhibition supports the conclusion that ADM is a substrate for NEP.

Neutral endopeptidase 24.11 (NEP) is a membrane-bound metalloprotease that cleaves endogenous peptides at the amino side of hydrophobic residues. This ectoenzyme is localized in greatest abundance in the kidney (9). NEP substrates include peptides such as atrial natriuretic peptide (ANP), bradykinin, and substance P (14). Indeed, inhibition of renal NEP has resulted in potentiation of the natriuresis secondary to exogenous administration of factors such as ANP (15). These studies underscore the importance of NEP as a regulator of sodium excretion. Although recent investigations have reported that the NH₂-terminal region of proADM is cleaved in vitro by NEP (16), to date, the pathway for degradation of mature ADM remains to be defined.

The current study was designed to test the hypothesis that NEP inhibition in vivo would potentiate the renal natriuretic actions of exogenous ADM and that systemic NEP inhibition would increase plasma ADM levels. We therefore investigated bilateral renal hemodynamic and excretory function in normal anesthetized dogs in which one kidney was exposed to locally increased concentration of ADM while the contralateral kidney served as control before and during acute systemic NEP inhibition. We also determined plasma ADM before and during systemic NEP inhibition.

METHODS

Study protocol. Studies were performed in six male mongrel dogs weighing between 20 and 24 kg. Dogs were maintained on a normal sodium diet with standard dog chow (Lab canine diet 5006; Purina Mills, St. Louis, MO) with free access to tap water. All studies conformed to the guidelines of the American Physiological Society and were approved by the Mayo Clinical Animal Care and Use Committee.

On the evening before the experiment, 300 mg of lithium carbonate was administered orally for the assessment of renal tubular function, and dogs were fasted overnight. On the day of the acute experiment, all dogs were anesthetized with pentobarbital sodium given intravenously (30 mg/kg). Supplemental nonhypotensive doses of pentobarbital sodium were given as needed during the experiment. After tracheal intubation, dogs were mechanically ventilated (Harvard res-
NEP INHIBITION POTENTIATES NATRIURETIC ACTIONS OF ADM

**RESULTS**

The bilateral renal responses to unilateral infusion of ADM before and during systemic NEP inhibition are reported in Table 1. In response to ADM infusion, GFR in the ADM-infused kidney did not change, whereas RBF increased from baseline and renal vascular resistance decreased compared with the control kidney.

Table 1. Renal hemodynamic and excretory function

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>ADM</th>
<th>ADM + NEPI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADM kidney</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>39.1 ± 4.6</td>
<td>38.8 ± 6.5</td>
<td>53.9 ± 9.9</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>198 ± 40</td>
<td>288 ± 56*</td>
<td>305 ± 59*</td>
</tr>
<tr>
<td>RVR, RU</td>
<td>0.91 ± 0.26</td>
<td>0.61 ± 0.15*</td>
<td>0.59 ± 0.14‡</td>
</tr>
<tr>
<td>UV, ml/min</td>
<td>0.20 ± 0.03</td>
<td>1.06 ± 0.17*‡</td>
<td>2.02 ± 0.36‡*</td>
</tr>
<tr>
<td>UNaV, µeq/min</td>
<td>41.2 ± 13.1</td>
<td>227.1 ± 40.9*‡</td>
<td>410.5 ± 79.1†‡</td>
</tr>
<tr>
<td>PFRNa, %</td>
<td>74.9 ± 5.5</td>
<td>52.6 ± 7.8*$</td>
<td>55.6 ± 7.5</td>
</tr>
<tr>
<td>DFRNa, %</td>
<td>97.7 ± 0.7</td>
<td>90.7 ± 1.2*$</td>
<td>87.2 ± 1.4$</td>
</tr>
<tr>
<td>UcAMP, pmol·ml⁻¹·min⁻¹</td>
<td>1,022 ± 167</td>
<td>1,196 ± 210</td>
<td>1,070 ± 183</td>
</tr>
<tr>
<td>UcGMP, pmol·ml⁻¹·min⁻¹</td>
<td>263 ± 76</td>
<td>125 ± 39*</td>
<td>145 ± 47*</td>
</tr>
<tr>
<td><strong>Control kidney</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>39.7 ± 3.7</td>
<td>36.3 ± 3.9</td>
<td>48.3 ± 4.8</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>189 ± 33</td>
<td>186 ± 32</td>
<td>177 ± 36</td>
</tr>
<tr>
<td>RVR, RU</td>
<td>0.87 ± 0.2</td>
<td>0.87 ± 0.2</td>
<td>0.99 ± 0.2</td>
</tr>
<tr>
<td>UV, ml/min</td>
<td>0.13 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>0.30 ± 0.10</td>
</tr>
<tr>
<td>UNaV, µeq/min</td>
<td>25.5 ± 13.6</td>
<td>32.5 ± 17.7</td>
<td>79 ± 37</td>
</tr>
<tr>
<td>PFRNa, %</td>
<td>78.3 ± 5.3</td>
<td>75.3 ± 4.3</td>
<td>72.2 ± 4.7</td>
</tr>
<tr>
<td>DFRNa, %</td>
<td>98.3 ± 0.6</td>
<td>97.9 ± 0.9</td>
<td>96.3 ± 1.5</td>
</tr>
<tr>
<td>UcAMP, pmol·ml⁻¹·min⁻¹</td>
<td>803 ± 91</td>
<td>804 ± 188</td>
<td>966 ± 195</td>
</tr>
<tr>
<td>UcGMP, pmol·ml⁻¹·min⁻¹</td>
<td>232 ± 33</td>
<td>187 ± 24</td>
<td>232 ± 42</td>
</tr>
</tbody>
</table>

Values are means ± SE. ADM + NEPI, ADM + NEP inhibition clearance; ADM, adrenomedullin; NEP, neutral endopeptidase 24.11; GFR, glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance; UV, urine flow; UNaV, urinary sodium excretion; PFRNa, proximal fractional reabsorption of sodium; DFRNa, distal fractional reabsorption of sodium; UcAMP, urinary cAMP excretion; and UcGMP, urinary cGMP excretion. *P < 0.05 vs. baseline. †P < 0.05 vs. ADM clearance. ‡P < 0.05 vs. control kidney.
Urine flow and urinary sodium excretion increased from baseline and were greater than that observed in the control kidney. Proximal and distal fractional reabsorption of sodium decreased in the ADM-infused kidney compared with the control kidney. There was no change in urinary cAMP excretion in either kidney, with no increase in urinary cGMP excretion.

Systemic NEP inhibition further increased urine flow and urinary sodium excretion in the ADM-infused kidney. This increase remained higher compared with the contralateral control kidney. Figure 1 illustrates the absolute change in urinary sodium excretion and urine flow during the ADM + NEPI clearance in both kidneys compared with the preceding clearance. Both urinary sodium excretion and urine flow were markedly potentiated by NEP inhibition in the ADM-infused kidney compared with the contralateral control kidney. In the control kidney with intrarenal infusion of saline vehicle, no change in any renal parameters were observed during the experiment.

Plasma ADM (Fig. 2) was unchanged during intrarenal ADM infusion but increased during systemic NEP inhibition. Urinary ADM excretion and mean arterial pressure remained unchanged throughout the experiment.

DISCUSSION

The current investigation was designed to test the hypothesis that systemic NEP inhibition potentiates the renal actions of the newly identified peptide ADM. The present findings confirm our hypothesis and report that NEP inhibition potentiates the renal natriuretic and diuretic responses to intrarenally administered ADM in normal anesthetized dogs. The increase in sodium excretion by NEP inhibition occurred in the absence of an increase in GFR or further increase in RBF, indicating that a decrease in tubular sodium reabsorption is the mechanism for natriuresis. The increase in plasma ADM during systemic NEP inhibition supports the conclusion that ADM is a substrate for NEP.

The current study confirms previous investigations that ADM is a natriuretic peptide (2, 4, 7, 22). In the present study, low intrarenal concentrations of ADM markedly increased sodium excretion in the absence of an increase in plasma or urinary ADM. This natriuretic response was associated with an increase in RBF in the absence of an increase in GFR. Therefore, alterations in peritubular physical factors secondary to renal vasodilatation may have resulted in decreasing peritubular gradient for sodium reabsorption. Alternatively, a direct tubular action of ADM is another mechanism that must be considered.

The studies have established that the biological actions of ADM may also involve activation of receptors coupled to adenylyl cyclase and with cAMP generation (3, 5). In the current investigation, however, we observed no increase in urinary cAMP excretion during intrarenal ADM infusion and systemic NEP inhibition. This lack of increase in urinary cAMP may be explained by two alternative mechanisms. The first is that the concentration of infused ADM was low and, when
infused intrarenally, had no effects on the contralateral kidney. Therefore, we may have activated adenylate cyclase receptors, but at a threshold level which did not result in the release of cellular cAMP into the urine. An alternative explanation is that nonadenylate cyclase receptors or nonadenylate cyclase mechanisms could also be involved in mediating the renal actions of ADM. This is a likely possibility based upon reports that suggest that the biological properties of ADM in the myocardium may also not be dependent upon activation of adenylate cyclase (21).

NEP is an ectoenzyme found in greatest abundance in the kidney, specifically in the brush-border vesicles of proximal tubules (9, 19). Repeated studies have established that other natriuretic peptides with disulfide rings such as ANP are degraded by NEP (9, 15, 23). In the current study, a significant increase in plasma ADM during systemic NEP inhibition supports the conclusion that ADM is an additional substrate for NEP. This conclusion is also supported by the study of Nagatomo et al. (16) in which proADM NH2-terminal 20 peptide was rapidly cleaved by NEP. In addition, Lewis et al. (11) reported that ADM is also degraded in vitro by ovine adrenal, lung, and kidney preparations and that this effect was prevented by metalloprotease inhibitors. In the latter study however, phosphoramidon, a specific inhibitor of NEP, did not alter ADM degradation (11). There are two important differences between the current study and that by Lewis et al. (11). First of all, different NEP inhibitors that may possess different potencies or specificities were used in the two studies. The additional variable is species, as the Lewis study was performed utilizing membrane preparations from ovine adrenal, kidney, and lung, whereas our investigation was performed in the intact canine. Therefore, the discrepancy between the two studies could be related to significant differences in the pharmacology of two NEP inhibitors or to the differences between the canine and ovine species.

The current study was designed to address the specific contribution of systemic NEP inhibition in potentiating the renal actions of ADM. We therefore administered ADM intrarenally at a concentration that did not result in a spillover to the other kidney or increase plasma levels so as to address the local actions of ADM independent of any changes in circulating hormones, such as ANP, which would have equally affected both kidneys. In the current study, sodium excretion was significantly potentiated only in the kidney that received ADM with no increase in urinary cGMP excretion. Such a response should exclude any contribution of ANP to the natriuretic response, as this natriuresis occurred only in the ADM-infused kidney during systemic inhibition of NEP with no increase in urinary cGMP excretion, a marker for ANP activation. We conclude that NEP inhibition specifically potentiates the natriuretic actions of ADM.

The current study has important therapeutic implications. First, intrarenal ADM administration was natriuretic and diuretic, and this action was potentiated by systemic NEP inhibition. Several studies have reported that NEP inhibition in heart failure and renal failure results in natriuresis (1, 12, 17, 18). Such natriuretic responses to NEP inhibition in cardiorenal disease states have been attributed to potentiation of ANP and kinins (12). The present study suggests that the natriuretic response to NEP inhibition in such disease states may also involve inhibition of ADM degradation. Based upon current findings, a role for ADM in the natriuretic response to NEP inhibition in these disease states should be considered.

In conclusion, the present investigation is the first to demonstrate that NEP inhibition potentiates the natriuretic and diuretic responses to intrarenal ADM. This potentiation occurs secondary to a decrease in tubular sodium reabsorption in association with renal vasodilation. Lastly, the increase in plasma ADM during systemic NEP inhibition supports the conclusion that ADM is a substrate for this ectoenzyme.

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