Effect of Atrial Natriuretic Factor on Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) Cotransport of Vascular Smooth Muscle Cells

NANCY E. OWEN, EUGENE N. BUSH, WILLIAM HOLLEMAN, AND MARTHA E. O'DONNELL

SUMMARY We previously demonstrated that vascular smooth muscle cells possess a prominent Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport system that can be markedly stimulated by elevations in levels of intracellular cyclic guanosine 3',5'-monophosphate (cGMP). Since others have shown that atrial natriuretic factor (ANF) can bind to specific membrane receptors and can enhance cGMP levels in vascular smooth muscle cells, we asked whether ANF could also stimulate Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport in vascular smooth muscle cells. It was discovered that rat atriopeptin III stimulated Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport of vascular smooth muscle cells in a concentration-dependent manner. In contrast, rat atriopeptin III had no effect on two other sodium transport systems known to be present in vascular smooth muscle cells (i.e., Na\(^{+}\)-H\(^{+}\) exchange and Na\(^{+}\)-K\(^{+}\)-adenosine triphosphatase (ATPase). These studies indicated that ANF selectively stimulates Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport of vascular smooth muscle cells. We then asked whether ANF-stimulated Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport was dependent upon the ability of ANF to enhance intracellular cGMP levels. When rat atriopeptin III-stimulated increases in cGMP were inhibited with the quinolinedione LY 83583, rat atriopeptin III could no longer stimulate Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport of vascular smooth muscle cells. Thus it appeared that the effects of ANF were dependent upon the ability of ANF to elevate intracellular cGMP levels. Finally, we asked whether ANF effects on Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport were related to the biological activity of ANF. Using vasorelaxation of histamine-constricted aortic strips as an index of biological activity, we observed a tight correlation between the biological activity of six ANF analogues and their ability to stimulate Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport and to elevate intracellular cGMP levels of vascular smooth muscle cells. It appears that ANF interacts with a specific membrane receptor to elevate intracellular cGMP, which then leads to stimulation of Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport. (Hypertension 10 [Suppl I]: M28-M30, 1987)

KEY WORDS • atrial natriuretic factor • Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport • cyclic GMP • vascular smooth muscle cells
TABLE 1. Effect of ANF on Na⁺-K⁺-Cl⁻ Cotransport of Vascular Smooth Muscle Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Na⁺-K⁺-Cl⁻ cotransport (mol K⁺ influx/g protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.23 ± 0.50</td>
</tr>
<tr>
<td>8-Br-cGMP</td>
<td>6.91 ± 0.28</td>
</tr>
<tr>
<td>Rat AP III</td>
<td>6.95 ± 0.49</td>
</tr>
<tr>
<td>LY 83583</td>
<td>2.19 ± 0.51</td>
</tr>
<tr>
<td>Rat AP III + LY 83583</td>
<td>2.27 ± 0.37</td>
</tr>
<tr>
<td>8-Br-cGMP + LY 83583 + M&amp;B 22,948</td>
<td>5.91 ± 0.92</td>
</tr>
</tbody>
</table>

Data are expressed as Na⁺-K⁺-Cl⁻ cotransport (bumetanide-sensitive K⁺ influx) and values are means ± SEM of triplicate measurements in Materials and Methods. Cells were preincubated for 5 minutes in HEPES-buffered minimal essential medium containing 10 M LY 83583 or vehicle. The medium was then replaced with identical fresh medium containing the agents to be tested. Concentrations were 8-Br-cGMP, 50 M; rat AP III, 100 nM; LY 83583, 10 M; and M&B, 100 M.

Results and Discussion

When VSM cells were challenged with increasing concentrations of rat AP III, rat AP III stimulated Na⁺-K⁺-Cl⁻ cotransport in a concentration-dependent manner. In six separate quadruplicate determinations, the concentration yielding half-maximal stimulation of cotransport (Kₐ) was 9 nM. This value was in close agreement with reported Kₐ values for ANF binding to specific membrane receptors of VSM cells and with reported Kₐ values for increasing intracellular cGMP in VSM cells.

The observation that ANF influences Na⁺-K⁺-Cl⁻ cotransport is in agreement with the findings of O'Grady et al. and of Solomon et al. These workers showed that ANF influences Na⁺-K⁺-Cl⁻ cotransport of teleost intestine and stimulates chloride secretion in shark rectal gland.

Subsequently, the effects of rat AP III on two other sodium transport systems was evaluated. It was found that rat AP III did not affect either Na⁺-H⁺ exchange or the Na⁺,K⁺-ATPase of VSM cells. This finding agrees with the work of others who have shown that ANF does not affect Na⁺,K⁺-ATPase activity of human erythrocytes or rat kidney. However, it disagrees with a published report demonstrating that ANF can inhibit Na⁺-H⁺ exchange in porcine kidney epithelial cells (LLC-PK). It is noteworthy that while the effects of ANF have been tested on three sodium transport systems, such measurements had not previously been carried out using a single cell type (e.g., VSM cells). Our findings suggested that rat AP III specifically stimulated Na⁺-K⁺-Cl⁻ cotransport of VSM cells, and probably did so through interaction with a specific membrane receptor.

These experiments established that ANF could stimulate Na⁺-K⁺-Cl⁻ cotransport and others had shown that ANF could stimulate elevations in intracellular cGMP levels; however, it was not known if these events occurred in series or in parallel. Because of this uncertainty, it was of interest to examine the relationship between ANF effects on Na⁺-K⁺-Cl⁻ cotransport and on cGMP levels in VSM cells. Thus we determined the effect of rat AP III on Na⁺-K⁺-Cl⁻ cotransport in the presence and absence of the quinolinedione, LY 83583; LY 83583 has been shown to block cGMP formation in a variety of tissues. It was found that Na⁺-K⁺-Cl⁻ cotransport occurring in the presence of 100 nM rat AP III was markedly reduced by 10 μM LY 83583 (see Table 1). Maximal inhibition was obtained with 10 μM LY 83583 and half maximal inhibition was seen at 0.3 μM LY 83583. It was found that the effects of LY 83583 could be reversed by addition of 8-Br-cGMP in the presence of a cGMP phosphodiesterase inhibitor. These findings demonstrated that when the ability of ANF to enhance intracellular cGMP levels was blocked, the ability of ANF to stimulate Na⁺-K⁺-Cl⁻ cotransport was inhibited. On this basis it was concluded that ANF stimulates Na⁺-K⁺-Cl⁻ cotransport in VSM cells through elevations in intracellular cGMP.

Although we provided strong evidence to suggest that ANF acted through cGMP to activate Na⁺-K⁺-Cl⁻ cotransport of VSM cells selectively, the biologi...
Biological Activity of ANF Analogues and Their Effect on Na\(^+\)-K\(^+\)-Cl\(^-\) Cotransport

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(K_D) for vasorelaxation (nM)</th>
<th>(K_D) for Na(^+)-K(^+)-Cl(^-) cotransport (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF (104–126)</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>ANF (103–126)</td>
<td>1.3</td>
<td>7.6</td>
</tr>
<tr>
<td>ANF (105–126)</td>
<td>5.1</td>
<td>75.9</td>
</tr>
<tr>
<td>[Asn(^{11})]ANF (103–126)</td>
<td>63.1</td>
<td>26.9</td>
</tr>
<tr>
<td>ANF (103–123)</td>
<td>81.3</td>
<td>63.1</td>
</tr>
<tr>
<td>[d-Ala(^{107})]ANF (105–121)</td>
<td>1.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Values are means ± SEM from four separate experiments. Vasorelaxation and Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport were measured as described in Materials and Methods. \(K_D\) is the concentration that produces 50% of the maximum response.

The biological relevance of ANF effects on sodium transport had not been determined. We assessed the biological activity of six ANF analogues by determining their ability to induce vasorelaxation in histamine-constricted aortic strips (Table 2). We then assessed the ability of these agents to stimulate Na\(^+\)-K\(^+\)-Cl\(^-\) co-transport of VSM cells. A very tight correlation was discovered between the biological activity (ability to induce vasorelaxation of aortic rings) and stimulation of Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport of VSM cells. Furthermore, the same potency order was noted for effects on intracellular cGMP levels (data not shown).

On the basis of these studies, it was concluded that ANF stimulation of Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport parallels biological activity. At present, the nature of this relationship is undefined. The only two known physiological roles of this cotransport are vectorial transport of salt and water in epithelia and volume regulation in single cells.\(^1\)–\(^3\) It is possible that some of the natriuretic and diuretic effects of ANF could be mediated through direct effects on the Na\(^+\)-K\(^+\)-Cl\(^-\) transporter. The identity of the relationship between ANF effects on Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport and ANF-mediated vasorelaxation remains to be determined.

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