SUMMARY  We previously demonstrated that vascular smooth muscle cells possess a prominent $\text{Na}^+\text{-K}^+\text{-Cl}^-$ 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 $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransport in vascular smooth muscle cells. It was discovered that rat atriopeptin III stimulated $\text{Na}^+\text{-K}^+\text{-Cl}^-$ 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., $\text{Na}^+\text{-H}^+$ exchange and $\text{Na}^+\text{-K}^+\text{-adenosine triphosphatase (ATPase)}$. These studies indicated that ANF selectively stimulates $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransport of vascular smooth muscle cells. We then asked whether ANF-stimulated $\text{Na}^+\text{-K}^+\text{-Cl}^-$ 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 $\text{Na}^+\text{-K}^+\text{-Cl}^-$ 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 $\text{Na}^+\text{-K}^+\text{-Cl}^-$ 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 $\text{Na}^+\text{-K}^+\text{-Cl}^-$ 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 $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransport. (Hypertension 10 [Suppl I]: M28-M30, 1987)
Few physiologically occurring agents have been shown to elevate intracellular cGMP levels. One agent that does have this activity is atrial natriuretic factor (ANF). \(^{12-15}\) ANF is a collective term for a group of bioactive peptides that interact at specific receptors \(^{16}\) to activate particulate guanylate cyclase. \(^{12,13}\) It has been demonstrated to cause natriuresis, diuresis, and vasorelaxation. \(^{19}\)

Although the complementary DNA sequence has been determined and it is well established that ANF is derived from a preprohormone, \(^{20}\) little is known about its mechanism of action. Because VSM cells possess specific high affinity ANF receptors and because ANF activates guanylate cyclase and causes an increase in cGMP levels in these cells, our laboratory investigated the effect of ANF on \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport of VSM cells. This report summarizes our studies and relates our work to other published reports concerning the effects of ANF on ion transport processes.

### Materials and Methods

Rat atriopeptin III (AP III) and other ANF analogues were generously provided by Dr. William Holleman (Abbott Laboratories, N. Chicago, IL, USA). 6-Anilino-5,8-quinolinedione (LY 83583) was provided by Lilly Research Laboratories (Indianapolis, IN, USA). M & B 22,948 (2-0-propoxypheynyl-8-aza-purin-6-one) was kindly provided by May and Baker Ltd. (Dagenham, Essex, United Kingdom). Bumetanide was the gift of Hoffmann-La Roche (Nutley, NJ, USA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

VSM cells were derived from explants of rat thoracic aorta as previously described. \(^{10,11}\) Measurements of \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport, \(^8\) \(\text{Na}^+\text{-H}^+\) exchange, \(^{21}\) \(\text{Na}^+\text{-K}^+\text{-adenosine triphosphatase (ATPase)}\) activity, \(^{22}\) intracellular cGMP levels, \(^{10,11}\) and vasorelaxation \(^{22}\) were carried out as described in previous publications.

### Results and Discussion

When VSM cells were challenged with increasing concentrations of rat AP III, rat AP III stimulated \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport in a concentration-dependent manner. In six separate quadruplicate determinations, the concentration yielding half-maximal stimulation of cotransport \((K_s)\) was 9 nM. This value was in close agreement with reported \(K_s\) values for ANF binding to specific membrane receptors of VSM cells \(^{16}\) and with reported \(K_s\) values for increasing intracellular cGMP in VSM cells. \(^{12,13}\) The observation that ANF influences \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport is in agreement with the findings of O'Grady et al. \(^{25}\) and of Solomon et al. \(^{24}\) These workers showed that ANF influences \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport of teleost intestine \(^{23}\) and stimulates chloride secretion in shark rectal gland. \(^{24}\)

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 \(\text{Na}^+\text{-H}^+\) exchange or the \(\text{Na}^+\text{-K}^+\text{-ATPase}\) of VSM cells. This finding agrees with the work of others who have shown that ANF does not affect \(\text{Na}^+\text{-K}^+\text{-ATPase}\) activity of human erythrocytes \(^{25}\) or rat kidney. \(^{26}\) However, it disagrees with a published report demonstrating that ANF can inhibit \(\text{Na}^+\text{-H}^+\) exchange in porcine kidney epithelial cells (LC-PK) \(^{37}\). 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 \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport of VSM cells, and probably did so through interaction with a specific membrane receptor.

These experiments established that ANF could stimulate \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) 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 \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport and cGMP levels in VSM cells. Thus we determined the effect of rat AP III on \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) 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. \(^{28}\) It was found that \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport occurring in the presence of 100 nM rat AP III was markedly reduced by 10 \(\mu\)M LY 83583 (see Table 1). Maximal inhibition was obtained with 10 \(\mu\)M LY 83583 and half maximal inhibition was seen at 0.3 \(\mu\)M 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 \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport was inhibited. On this basis it was concluded that ANF stimulates \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport in VSM cells through elevations in intracellular cGMP.

Although we provided strong evidence to suggest that ANF acted through cGMP to activate \(\text{Na}^+\text{-K}^+\text{-Cl}^-\) cotransport of VSM cells selectively, the biologi-
Table 2. Biological Activity of ANF Analogues and Their Effect on Na\(^+\)-K\(^+\)-Cl\(^-\) Cotransport

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(K_{50}) for vasorelaxation (nM)</th>
<th>(K_{50}) 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(^{17})]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. \(K_{50}\) is the concentration that produces 50% of the maximum response.

4. Garay RP. Inhibition of Na/K cotransport system by cyclic AMP and 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. 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 demonstrated.

References

4. Garay RP. Inhibition of Na/K cotransport system by cyclic AMP and intracellular calcium in human red cells. Biochim Biophys Acta 1982;688:786–792
Effect of atrial natriuretic factor on Na+-K+-Cl- cotransport of vascular smooth muscle cells.

N E Owen, E N Bush, W Holleman and M E O’Donnell

_Hypertension_. 1987;10:I128
doi: 10.1161/01.HYP.10.5_Pt_2.I128

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1987 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/10/5_Pt_2/I128

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/