Atrial natriuretic peptide clearance receptor participates in modulating endothelial permeability

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HempeL, Albrecht, Thomas Noll, Christoph Bach, Hans Michael Piper, Roland Willenbrock, Klaus Höhnel, Hermann Haller, and Friedrich C. Luft. Atrial natriuretic peptide clearance receptor participates in modulating endothelial permeability. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1818–H1825, 1998.—The atrial natriuretic peptide (ANP)-C receptor is generally believed to clear ANP; however, the ANP-C receptor may serve to reduce cAMP by inhibiting adenylyl cyclase. ANP decreases endothelial permeability in coronary endothelial cell monolayers. We tested the hypothesis that part of this effect might be mediated by the ANP-C receptor. We used an endothelial cell monolayer from rat coronary endothelium and measured albumin flux. We applied either ANP or a ring-deleted ANP (C-ANP), which only stimulates the ANP-C receptor. ANP and C-ANP both decreased permeability from 100 pM to 100 nM by 60 and 30%, respectively. ANP increased endothelial cGMP contents 5.5-fold, whereas C-ANP had no effect. ANP reduced endothelial cAMP contents by 75%, which was only partly blocked by pertussis toxin. C-ANP also reduced cAMP; however, this effect was completely blocked by pertussis toxin. Protein kinase G inhibition blocked the ANP-mediated decrease in permeability by 50%. In contrast, pretreatment with pertussis toxin, in the face of protein kinase G inhibition, blocked the effect completely. C-ANP decreased permeability by half the amount of ANP. This C-ANP effect was completely blocked by pertussis toxin but not by protein kinase G inhibition. Isoproterenol (10 µM) increased permeability by almost 50%, which was completely blocked by ANP but only partially blocked by C-ANP. The C-ANP effect was blocked completely by pertussis toxin. Isoproterenol increased cAMP threefold, which was abolished by ANP. C-ANP reduced the isoproterenol-induced increase in cAMP by 50%. Isoproterenol had no effect on cGMP. We conclude that agonist binding to the ANP-C receptor inhibits cAMP production via a G protein-coupled signaling system. This inhibition may contribute to the decreased endothelial permeability evoked by ANP in this system.

atrial natriuretic peptide receptors; endothelial permeability; cGMP; cAMP

Atrial natriuretic peptide (ANP) promotes natriuresis and diuresis (26), inhibits renin and aldosterone release (8), and has important effects on endothelial cell function (28, 30, 31). The effect on endothelial cell permeability is variable and dependent on endothelial cell origin (19, 46, 48). ANP induces an increase in hematocrit in rats, even if they are splenectomized and nephrectomized (16, 44, 47). In endothelial cell monolayers from the bovine pulmonary artery, ANP increased permeability via transcellular pathways (6, 46). In monolayers of rat coronary endothelial cells on the other hand, ANP reduced permeability while increasing cGMP and reducing cAMP (16). ANP effects are mediated by specific receptors. The ANP-A receptor and the ANP-B receptor are both coupled to guanylyl cyclase and produce cGMP (11, 12, 37). cGMP is an activator of a cGMP-dependent protein kinase (14), which is intimately involved in mediating the ANP-induced effects on endothelial cell layer permeability (17). The ANP-C receptor is particularly abundant in the kidney and is believed to be primarily responsible for clearing ANP from the circulation (1, 29). However, not all authorities accept that ANP-C receptors are silent. Stimulation of these receptors has been reported to inhibit adenylyl cyclase activity in platelets and pheochromocytoma cells (2, 15), inhibit neurotransmission in autonomic nerve terminals (15, 23), inhibit proliferation of cultured rat aortic smooth muscle cells (10), and increase inositol phosphate in vascular smooth muscle cells (20). Furthermore, ANP was shown to inhibit the production and secretion of endothelin from cultured endothelial cells via the ANP-C receptor (21). C-ANP is a ring-deleted ANP that only binds to the ANP-C receptor (3). All three receptors have been described in endothelial cells (1). We studied the role of the ANP-C receptor in rat coronary endothelial cells by using the ring-deleted C-ANP as a probe, and we compared the effects of ANP with those of C-ANP. We found that ANP-C receptor occupancy induces detectable effects on endothelial barrier function and that these effects rely on a pertussis toxin-sensitive, G-protein-coupled signaling system distinct from that utilized by the ANP-A and ANP-B receptors.

MATERIALS AND METHODS

Materials. Falcon plastic tissue culture dishes were obtained from Becton Dickinson (Heidelberg, Germany); Transwell polycarbonate filter inserts (24-mm diameter, 0.4-µm pore size) were from Costar (Bosheim, Germany); fetal bovine serum (FBS), newborn calf serum (NCS), medium 199, penicillin-streptomycin, and trypsin-EDTA were from Gibco (Eggenstein, Germany); ANP, C-ANP, KT5823, and pertussis toxin were from Calbiochem (Bad Soden, Germany); and trypsin blue, fatty acid-free albumin, and isoproterenol were obtained from Sigma (Deisenhofen, Germany). All other chemicals were of the best available quality, usually analytic grade.

Cell cultures. Coronary endothelial cells were isolated from 250-g male Wistar rats and grown in culture as previously described (35). Briefly, hearts were perfused with collagenase, chopped, and dissolved into a suspension. From this suspen-
sion, the fraction of endothelial cells was purified. Cells were plated at a density of 10^6 cells on 100-mm plastic petri dishes. The cells were cultured at 37°C in medium 199 with Earle’s salt, supplemented with 100 IU/ml penicillin G, 100 µg/ml streptomycin, and 10% (vol/vol) NCS and 10% (vol/vol) FBS. The medium was renewed every second day. After 5 days, when the cells had grown to confluence, they were trypsinized in phosphate-buffered saline composed of (mM) 137 NaCl, 2.7 KCl, 1.5 KH2PO4, and 8.0 Na2HPO4, at pH 7.4, with 0.05% (wt/vol) trypsin and 0.02% (wt/vol) EDTA and seeded at a density of 7 × 10^4 cells/cm² on either 24-mm round polycarbonate filters or 60-mm plastic petri dishes for determination of albumin flux, CAMP, and cGMP contents, respectively.

Experiments were performed with confluent monolayers, 4 days after the cells were seeded on filters. As previously reported (18, 19, 35), the purity of these cultures was >97% endothelial cells. The remaining cell types in this preparation are primarily pericytes. Contamination with vascular smooth muscle cells is not a factor in this preparation (35).

Macromolecule permeability. The permeability across the endothelial cell monolayer was studied in a two-compartment system separated by a filter membrane (36, 38). Both compartments contained modified Tyrode solution [composition of (mM) 150 NaCl, 2.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 1.0 CaCl2, 0.05 K2HPO4, 8.0 Na2HPO4, and 30.0 HEPES (pH 7.4, 37°C)] supplemented with 10% (vol/vol) NCS and 10% (vol/vol) FBS. There was no hydrostatic pressure gradient between both compartments. The “luminal” compartment containing the monolayer had a volume of 2.5 ml, and the “abluminal” had a volume of 10.5 ml. The fluid in the abluminal compartment was constantly stirred. Trypan blue-labeled albumin (50 µM) was added to the luminal compartment. We precipitated the trypan blue-labeled albumin with HCl and determined that no trypan blue was present in the supernatant by means of photometry with two wavelengths. We were thus assured of adequate binding to the albumin preparation. We had earlier employed fluorescein isothiocyanate-labeled albumin (45) and compared this method with trypan blue-albumin labeling. We found these methods to give the same results. The appearance of trypan blue-labeled albumin in the abluminal compartment was continuously monitored by pumping the liquid through a two-wavelength photometer (Spectra 510, Zeiss; Jenaval, Germany; wavelength for measurement of trypan blue 580 nM, control wavelength 720 nM). Increases of the concentration of trypan blue-labeled albumin were detected with a time delay of <15 s. The concentration of trypan blue-labeled albumin was determined in the abluminal compartment after 10 min of incubation. The concentration did not change significantly in the time frame of the experiments.

The albumin flux (F, expressed as mol·s⁻¹·cm⁻²) across the monolayer with the through the surface area S was determined from the rise of albumin concentration ([A]₂) during the time interval Δt in the abluminal compartment (V, volume): F = ([A]₂ − [A]₁)/Δt × V/S, where t is time. To facilitate the comparison of data obtained in the present study with those of other studies, the permeability coefficient (P, expressed as cm/s) (38) of the combined system of monolayer and filter support can be calculated from F as described in equation (2) of the text of reference 19:

\[ P = \frac{F}{(\beta_1 - \beta_2)} \times \frac{1}{V} \]

where \([A]_1\) and \([A]_2\) denote tracer concentrations in the luminal and abluminal compartments, respectively. Because the driving force \([\beta_1] - [\beta_2]\) only changed within the course of the described experiments, the relative changes in F correspond to similar changes in P. Values of F were expressed as a percentage of a control situation. The F data are a percentage of control; 100% corresponds to albumin flux of 4.2 ± 0.4 × 10⁻¹³ mol·s⁻¹·cm⁻² and a permeability coefficient of 5.37 ± 0.5 × 10⁻⁶ cm²/s of barrier formed by endothelial monolayer and filter support (32).

We used pertussis toxin (32), KT5823, and isoproterenol (45) in our experiments at doses based on dose-response curves obtained in separate preliminary experiments. We selected these doses providing a maximum effect at a minimum concentration (data not shown). Pertussis toxin was applied 2 h before the experiments. The monolayers were then washed by a threefold medium change immediately before the experiments (32). KT5823 was applied 30 min before the experiments.

Experimental protocols. The basic medium used in these incubations was modified Tyrode solution as described in Macromolecule permeability (pH 7.4 at 37°C) with 5% (vol/vol) NCS and 5% (vol/vol) FBS. Determination of macromolecule permeability and of cGMP and cAMP contents of the endothelial monolayers was started after an initial equilibration period of 30 min, and then the basal albumin flux and cGMP and CAMP contents of each monolayer were determined for another 30 min of incubation. In one set of experiments, monolayers were preincubated for 2 h in the presence of 1 µg/ml pertussis toxin and then washed by a threefold medium change. Afterwards, agents were added as indicated, and the response of the albumin flux and cGMP and CAMP contents were determined for an additional 30 min.

The time course of the ANP-related effects on the endothelial monolayer was determined in these studies in separate experiments over 1 h and was similar to what we have observed previously. We observed that the permeability effects were at a maximum by 30 min. The effects on cGMP and CAMP were at a maximum by 10 min (data not shown). Thus the functional permeability measurements were all made at 30 min, whereas the second messengers were determined at 10 min in separate cell experiments. The second messenger adjustments preceded the functional effects as shown by ourselves and others (19, 32, 45).

Extraction and assay of cellular cGMP and CAMP contents. At the end of the incubations, the incubation medium of the monolayer cultures was aspirated, ice-cold ethanol was added to terminate the reactions, and the petri dishes were stored at -80°C. To determine the intracellular cGMP and CAMP contents, the ethanol was evaporated at 60°C, and the samples were suspended in double-distilled water, transferred into Eppendorf reaction tubes, and centrifuged for 5 min at 14,000 g. cGMP and CAMP concentrations in the supernatants were determined using radioimmunoassays (Amersham, Braunschweig, Germany). The protein contents of the samples were determined according to Bradford (7) using bovine serum albumin as the standard.

Reverse transcriptase-polymerase chain reaction for ANP receptors. RNA was extracted from the cardiac endothelial cells by the method of Chomczynski and Sacchi (13). Isolated RNA (800 ng) were reverse transcribed into cDNA by Moloney murine leukemia virus reverse transcriptase in a volume of 100 µl. CDNA (10 µl) was used to detect ANP-A, ANP-B, ANP-C, and GAPDH, respectively in a PCR reaction by using specific primer pairs. Ten microliters of 100 µl-PCR product were loaded per lane and resolved in a 1.5% agarose gel.

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Statistical analysis. Data are given as means ± SD with n equaling six experiments using independent cell preparations. Statistical analysis of data was performed according to Student’s unpaired t-test. P values of <0.05 were considered significant.

RESULTS

Figure 1 shows the effect of ANP (Fig. 1A) and C-ANP (Fig. 1B) on albumin flux through the endothelial
Figure 1. A: effect of atrial natriuretic peptide (ANP) on albumin flux in endothelial cell monolayer. A dose-related decrease in permeability is observed up to 100 nM. At 1 µM, the effect is blunted. B: effect of ring-deleted ANP (C-ANP) on albumin flux in endothelial cell monolayer. A dose-related decrease in permeability is observed up to 100 nM. At 1 µM, the effect is blunted. Thus ANP and C-ANP exert similar effects on permeability, although that of ANP is stronger. 100% corresponds to permeability of 5.37 ± 0.5 × 10⁻⁶ cm²/s of barrier formed by endothelial monolayer and filter support. Data are means ± SD after 30 min of incubation of n = 6 separate experiments. *P < 0.05 vs. control (C).

Table 1. Dose-dependent changes in cGMP and cAMP in endothelial cells in response to ANP or C-ANP (10 min)

<table>
<thead>
<tr>
<th>Condition</th>
<th>cAMP, pmol/mg protein</th>
<th>cGMP, pmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.71 ± 0.21</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>ANP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 µM</td>
<td>0.62 ± 0.28*</td>
<td>2.11 ± 0.25*</td>
</tr>
<tr>
<td>100 nM</td>
<td>0.40 ± 0.21*</td>
<td>2.22 ± 0.22*</td>
</tr>
<tr>
<td>10 nM</td>
<td>0.95 ± 0.25*</td>
<td>1.31 ± 0.25*</td>
</tr>
<tr>
<td>1 nM</td>
<td>1.18 ± 0.27</td>
<td>0.60 ± 0.21</td>
</tr>
<tr>
<td>100 pM</td>
<td>1.35 ± 0.29</td>
<td>0.56 ± 0.19</td>
</tr>
<tr>
<td>C-ANP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 µM</td>
<td>1.12 ± 0.22*</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>100 nM</td>
<td>0.99 ± 0.21*</td>
<td>0.43 ± 0.19</td>
</tr>
<tr>
<td>10 nM</td>
<td>1.12 ± 0.22*</td>
<td>0.40 ± 0.17</td>
</tr>
<tr>
<td>1 nM</td>
<td>1.31 ± 0.24</td>
<td>0.41 ± 0.19</td>
</tr>
<tr>
<td>100 pM</td>
<td>1.40 ± 0.23</td>
<td>0.42 ± 0.18</td>
</tr>
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Values are means ± SD; n = 6 separate experiments. ANP, atrial natriuretic peptide; C-ANP, ring-deleted ANP. *P < 0.05 vs. control.
KT5823 and pertussis toxin totally eliminated the ANP-induced decrease in monolayer permeability. C-ANP (Fig. 3B) alone decreased albumin flux by 30%. This effect was not influenced by KT5823. However, in the presence of pertussis toxin, C-ANP no longer had an effect on endothelial permeability. These results indicate that the decrease in endothelial cell layer permeability induced by ANP is in part dependent on protein kinase G as well as on the function of a Gi protein responsible for reducing cAMP production.
We next performed a positive control experiment with isoproterenol (10 µM), as shown in Fig. 4. β2-Adrenoceptor stimulation is known to increase endothelial cell layer permeability in rat coronary endothelial cells via increased cAMP levels by activation of adenylate cyclase (19). The increase in permeability induced by isoproterenol (Fig. 4A) was abolished by ANP (100 nM). C-ANP (100 nM) reduced the isoproterenol-induced increase by 50%. Isoproterenol increased cAMP threefold. ANP abolished this increase completely. C-ANP reduced the increase in cAMP by 50%. Isoproterenol alone had no effect on cGMP. ANP (Fig. 4B) increased cGMP 5.5-fold, whereas C-ANP had no effect on cGMP. These results suggest that ANP influences endothelial cell layer permeability via two distinct pathways. One pathway involves cGMP production, whereas the other pathway involves cAMP reduction.

Finally, we verified the presence of all three ANP receptors, namely, the ANP-A, ANP-B, and ANP-C receptors, on rat coronary endothelial cells. We used RT-PCR, and as can be seen in Fig. 5, all three receptor mRNAs were expressed in our cell preparation.

DISCUSSION

The important findings in this study were that the ANP-induced decrease in permeability of coronary artery endothelial cell monolayers may be mediated in part by the ANP-C receptor. The ANP-C receptor-mediated effect did not involve the production of cGMP but instead featured a reduction in cAMP. This reduction relied on a pertussis toxin-sensitive, G protein-coupled signaling system distinct from that utilized by the ANP-A and ANP-B receptors. We also showed that the effect on permeability mediated by the ANP-A and ANP-B receptors was dependent on the action of protein kinase G. Inhibiting this enzyme blunted the ANP-mediated decrease in permeability, whereas inhibiting both protein kinase G and G protein-coupled signaling abolished the effect completely. The decrease in permeability mediated by the ANP-C receptor was independent of protein kinase G. We included a positive control experiment in that we showed that ANP abolishes the increase in endothelial monolayer permeability induced by isoproterenol. The C-ANP analog, which only stimulates the ANP-C receptor, decreased the isoproterenol effect by 50%, again without the generation of cGMP. Our current results extend our earlier observations that cGMP and cAMP are functional antagonists in the control of macromolecular permeability of coronary endothelial monolayers (19). Here we
show that activation of the ANP-C receptor leads to a reduction in cAMP, whereas activation of the ANP-A and ANP-B receptors leads to an increase in cGMP. Both effects work in concert to decrease the monolayer permeability.

We demonstrated that the ANP-C receptor is present on coronary endothelial cells and showed that the ANP-C receptor is of functional significance in these cells. Anand-Srivastava et al. (3) studied rat aorta, brain striatum, anterior pituitary tissue, and adrenal cortical membranes. They utilized the same ring-deleted analog of ANP, which is only capable of stimulating the ANP-C receptor. In all of these tissues, they showed that stimulation of this receptor reduced cAMP generation by inhibiting adenylate cyclase. Furthermore, they documented that the effect was pertussis toxin sensitive, implicating an inhibitory guanine nucleotide regulatory protein. We are not the first to show that the ANP-C receptor is present on endothelial cells. Hu et al. (21) showed that ANP in doses similar to those that we employed inhibits the production and secretion of endothelin from cultured endothelial cells from bovine aorta. Pedram et al. (34) recently demonstrated that natriuretic peptides inhibit endothelin-stimulated vascular endothelial cell growth factor (VEGF) production. The authors concluded that the ANP-C receptor was responsible for mediating this effect because C-ANP, which binds only to this receptor, was equally effective as ANP in this regard. Furthermore, the ANP-mediated effect was not influenced by administration of a cGMP inhibitor. Hutchinson et al. (22) presented strong evidence that the ANP-C receptor is important in ANP-induced growth inhibition of vascular smooth muscle cells. The importance of the ANP-C receptor to the vascular wall was further shown by Kishimoto et al. (24), who found that this receptor is transcriptionally downregulated by β2-adrenergic stimulation in vascular smooth muscle cells. These observations are of interest from the fact that a β2-adrenergic agonist increased endothelial permeability in our model, an effect that was attenuated in part by occupancy of the ANP-C receptor.

We are aware that our in vitro model, showing that ANP induces a tightening or a lessened permeability of a cultured endothelial cell layer, rather than increasing endothelial barrier permeability, is a conundrum. Battle et al. (6) showed earlier that ANP increases the permeability of endothelial cells harvested from the pulmonary artery and that the effect involves the generation of cGMP. They further demonstrated that the increase in permeability involved membrane folding by light and scanning electron microscopy. Stelzner et al. (40) also studied pulmonary artery endothelial cell monolayers and found that by increasing cAMP concentrations, albumin transfer was decreased. Yonemaru et al. (48) studied unstimulated endothelial cell layers from bovine pulmonary artery endothelial cells and reported similar results. We have preliminary observations from pulmonary artery endothelial cells in accord with these findings. Nevertheless, in agonist-stimulated endothelial cell layers, with bradykinin or thrombin, for example (46), an increase in cGMP production serves to restore the endothelial barrier function to normal.

Tucker et al. (43) examined blood-tissue albumin transport after physiological ANP infusions in rats. They observed that filtration-dependent, tissue-selective increases in albumin transport occurred. Increased albumin clearance was shown in small intestine, colon, fat, kidney, and skeletal muscle; however, no increase was observed in skin, diaphragm, or lung. In the heart, an increase was observed only in the left ventricle at a high ANP infusion dose. Tucker (42) verified that endogenous ANP was responsible by performing atrial appendectomy in rats. We concentrated our interests on coronary artery endothelial cells, because we are particularly interested in coronary disease. The permeability decreases after ANP that we observed in the current study are in line with our previous report and also concur with endothelial monolayer studies of Wessendorp et al. (46). We believe that endothelial cells from various tissue may differ in regard to ANP responses. Watanabe et al. (45) found that adenosine both increased and decreased endothelial monolayer permeability to protein. The direction was related to the origin of the cells. Sill et al. (39) showed that shear stress elevated monolayer permeability and found that dibutyl cAMP and phosphodiesterase inhibition blocked the effect. On the other hand, Kubes and Granger (25) observed an increase in the permeability index in cat and rat mesentery during nitric oxide synthesis blockade. Finally, Lofton et al. (27) found that glucose oxidase-induced increases in endothelial monolayer permeability to albumin were inhibited by pretreatment with ANP in doses similar to those we employed. The authors found that ANP reduced the changes in cell shape provoked by the oxidant. We believe that extrapolating these in vitro monolayer data to whole organs or organisms requires appropriate caution, particularly because we administered ANP in a pharmacological fashion and we are unable to state for certain what the meaning of our doses is in terms of ANP levels found in the circulation of living organisms.

We understand that extrapolating permeability of culture systems to an in vivo situation is problematic. The permeability of cultured monolayers is several orders of magnitude greater than that of intact microvessels (35). We nevertheless believe that our findings may be of clinical significance. Various natriuretic peptide genes are activated in failing and ischemic ventricles, including ANP (4). Takahashi et al. (41) provided data showing that the ventricular expression of B-type ANP, C-type ANP, and ANP mRNAs is concordantly regulated in patients with severe congestive heart failure. They suggested that this coexpression of these two natriuretic peptides may play a role in compensatory processes in heart failure; however, they did not go into detail on how natriuretic peptides may benefit failing or ischemic myocardium. We showed earlier that ANP protects against reoxygenation-induced hypercontracture in cardiomyocytes, suggesting a protective role of ANP in the presence of ischemia (18). Furthermore, Noll et al. (33) also found that
depriving coronary endothelial monolayers of energy (ischemia) increases endothelial cell-layer permeability. Thus a second important protective effect of ANP may be related to the results reported here. Under the circumstances of ischemia and/or ventricular dysfunction, a decrease in endothelial permeability may assist in avoiding intercellular edema, increasing the barrier to \( \text{O}_2 \) and \( \text{CO}_2 \) exchange, and maintaining endothelial barrier function. Whereas the protective effects from ischemia in cardiomyocytes (18) appeared to depend exclusively on the generation of cGMP, the endothelial effects in the heart rely on both cGMP generation and cAMP inhibition.

That the inhibition of cAMP leads to a decrease in coronary endothelial cell-layer permeability was also shown in an earlier study involving neuropeptide Y (32). In that study, the neuropeptide Y effects were also influenced by pertussis toxin, implicating a Gi protein-sensitive pathway. Neuropeptide Y is released by adrenergic nerve endings that service the coronary arteries. The effects mediated by the ANP-C receptor were similar and suggest that neuropeptide Y and ANP may act in concert to reduce endothelial layer permeability in the heart. The importance of the cAMP downregulation was underscored by our experiments with isoproterenol. This agonist alone markedly stimulated adenylate cyclase and increased cAMP production in endothelial cells, resulting in increased permeability (19, 32). Cotreatment with C-ANP suppressed the isoproterenol-related effects by 50% and reduced the cAMP response accordingly. ANP suppressed the isoproterenol-related increase in permeability completely, indicating the additional role of cGMP generation in this process. The magnitude of the cGMP effect was demonstrated by the experiments involving the inhibition of protein kinase G, which abolished the cGMP-related effects.

Our data underscore the heterogeneity of endothelial cell function and the complexity of ANP-related effects on the endothelial barrier. In the heart, ANP appears to maintain the integrity of the endothelial barrier, both via a generation of cGMP, which is mediated by the ANP-A and ANP-B receptors, and via a decrease in cAMP, which is mediated by the ANP-C receptor. Our results implicate a role for the ANP-C receptor, which is not only present on endothelial cells but also capable of mediating important physiological effects. We suggest that this receptor is responsible for much more than merely clearing superfluous ANP from the circulation.

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