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Atrial natriuretic peptide degradation by CPA47 cells: evidence for a divalent cation-independent cell-surface proteolytic activity

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Atrial natriuretic peptide (ANP) is rapidly cleared and degraded in vivo. Nonguanylate-cyclase receptors (C-ANPR) and a metalloproteinase, neutral endopeptidase (EC 3.4.24.11) (NEP 24.11), are thought to be responsible for its metabolism. We investigated the mechanisms of ANP degradation by an endothelial-derived cell line, CPA47. CPA47 cells degraded 88% of ^{125}I-ANP after 1 h at 37°C as determined by HPLC. Medium preconditioned by these cells degraded 41% of the ^{125}I-ANP, and this activity was inhibited by a divalent cation chelator, EDTA. Furthermore, a cell-surface proteolytic activity degraded ^{125}I-ANP in the presence of EDTA when receptor-mediated endocytosis was inhibited either by low temperature (4°C) or by hyperosmolarity at 37°C. The metalloproteinase, NEP 24.11, is unlikely to be the cell-surface peptidase because ^{125}I-ANP is degraded by CPA47 cells at 4°C in the presence of 5 mM EDTA. These data indicate that CPA47 cells can degrade ANP by a novel divalent cation-independent cell-surface proteolytic activity.

Introduction

Atrial natriuretic peptide (ANP) is a fluid-regulating peptide hormone that promotes vasorelaxation, natriuresis, and diuresis and inhibits pressor and volume-conserving hormones [1]. ANP, a 28 amino acid peptide, is the C-terminal cleavage product of the 126 amino acid prohormone. ANP contains a 17-member ring created by an intradisulfide bond and has only one tyrosine at the C-terminal of the molecule [1]. The synthesis and secretion of ANP is increased as a result of elevated intracardiac pressure and increased tension of the atrial wall [1]. The mechanisms for release and for clearance of ANP from the circulation play crucial roles in modulating this hormone's physiologic effects. ANP is cleared rapidly from the bloodstream of rats (15 to 31 s) [2] and humans (less than 5 min) [3]. The removal and degradation of ANP is believed to result from two mechanisms: (1) endoproteinase activity [4–6]; and (2) ligand binding to the nonguanylate cyclase-linked ANP receptors (C-ANPR) [7–10].

Koehn and co-workers [11] reported that an enzyme derived from cortex membranes of rat kidney has a K_m of 10 μM for the metabolism of ANP and that this activity is inhibited by EDTA. Further studies using inhibitors of endoproteinase (CEC 3.4.24.11) (NEP 24.11) suggest that this enzyme is responsible for ANP degradation in pig kidney microvillar membranes [12]. In another study, inhibitors of NEP 24.11 were included in intravenous infusions of ANP [6]. Prolonged biological effects of ANP were observed, indicating that NEP 24.11 was involved in ANP degradation in vivo. Other investigators have reported that aprotinin could inhibit ANP degradation in vivo and that aprotinin-sensitive kallikrein isolated from human urine could degrade ANP [4,5].

In addition to the enzymatic degradation of ANP in kidney tissues, C-ANPR have been postulated to be involved in ANP degradation [9]. Adding a peptide analogue of ANP (ANP 4–23; C-ANP) that competes for ANP binding to the C-ANPR was shown to inhibit ANP degradation in vivo [9]. Furthermore, peptide analogs of ANP that are fully resistant to the action of NEP 24.11 in vitro were cleared from the rat circula-

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Abbreviations: ANP, atrial natriuretic peptide; NEP 24.11, neutral endopeptidase (EC 3.4.24.11); BAEC, bovine aortic endothelial cells; SMC, smooth muscle cells; CTC, cultured thyroid cells; BM, binding medium; BSA, bovine serum albumin; CM, conditioned medium.

tion at the same rate as was ANP [13]. These data suggest that C-ANPR are responsible for the rapid clearance of intact ANP from the bloodstream [9,13].

Recent studies on the cellular mechanisms of ANP degradation by vascular smooth muscle cells (SMC), bovine aortic endothelial cells (BAEC), and cultured thyroid cells (CTC) suggest that receptor-mediated endocytosis of C-ANPR is one mechanism by which cells can internalize and degrade ^{125}I-ANP [10,14–16]. However, Johnson and co-workers [14] reported only a partial inhibition of ^{125}I-ANP degradation into ^{125}I-Tyr in the presence of excess nonlabeled ANP and lysosomotropic agents, suggesting that SMC could have another mechanism for ^{125}I-ANP degradation.

BAEC also appear to degrade ^{125}I-ANP by receptor-mediated endocytosis [15]. However, BAEC were reported to bind only 4 fmol of ^{125}I-ANP on their surface yet these cells degraded 117 fmol of radiolabeled AMP in 20 min at 37°C. This would require BAEC to recycle their surface receptors about 30 times in 20 min (once every 40 s). This is an extremely fast rate of receptor recycling compared with a typical type 1 pathway for receptor-mediated endocytosis, which requires 10–20 min for receptor recycling, or compared with ^{125}I-ANP degradation by SMC, in which 1 h is necessary to degrade all its surface-bound ^{125}I-ANP [10,17]. Furthermore, lysosomotropic agents only delayed ^{125}I-ANP degradation into ^{125}I-Tyr and Arg-^{125}I-Tyr and did not cause a significant increase in intracellular ^{125}I-ANP during the first 10 min [15]. Neutralization of acidic compartments should inhibit most ligand degradation and cause a significant increase in intracellular accumulation of the ligand (i.e., ^{125}I-ANP degradation by SMC) [10,14]. Thus, data reported by Johnson and co-workers [15] are not consistent with ^{125}I-ANP degradation by only a receptor-mediated process. Johnson and Foster [18] reported the presence of an alternative extracellular pathway that degrades ^{125}I-ANP into a C-terminal tripeptide in BAEC.

CTC also appear to have mechanisms other than receptor-mediated endocytosis for the degradation of ^{125}I-ANP [16]. Although receptor-mediated endocytosis occurs in CTC, excess unlabeled ANP, which competes for radiolabeled ANP binding to C-ANPR, did not effectively block ^{125}I-ANP degradation [16]. Thus, these previous reports suggest that cellular mechanisms other than receptor-mediated endocytosis may be involved in the degradation of ^{125}I-ANP.

The purpose of our study was to determine if a cell-surface proteolytic activity was an alternative mechanism for the degradation of ANP on endothelial cells. Endothelial cells line the vasculature and are therefore intimately involved with the regulation of and responsiveness to circulating hormones. Furthermore, recent studies have demonstrated the presence of cell-surface peptidases on endothelial cells that degrade biologically active peptides such as substance P, pancreatic polypeptide and neuropeptides [19,20]. Thus, endothelial cells would likely have a cell-surface peptidase to degrade ANP.

Materials and Methods

Materials

Human ANP was purchased from Peninsula Laboratories (Belmont, CA). Fetal calf serum and bovine calf serum were obtained from Hyclone (Logan, UT). ^{125}I-Human ANP (specific activity = 2000 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Burdick and Jackson HPLC solvents were acquired from Baxter Scientific (McGraw Park, IL). All other chemicals were purchased from Sigma (St. Louis, MO).

CPA47 cell culture

CPA47 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and were cultured according to ATCC instructions with the exception that cells were trypsinized when passaged. Cells were not used after passage 40 (i.e., 13 passages after obtaining the cells from ATCC). Cells were seeded at a density of $(1.7–2.7)\cdot 10^4$ cells/cm^2 in 12-well plates and used in subsequent experiments within 5 days after subculturing.

Kinetics of ^{125}I-ANP binding to CPA47 cells

The kinetics of ^{125}I-ANP binding to CPA47 cells was studied at 37°C. CPA47 cells were grown to approx. 90% confluence. Cells were then washed with binding medium (BM, 1:1 ratio of F-12 (Sigma No. N 6760) and Minimal Essential Medium (Sigma No. M 0643) containing 6 g/l Hepes and 4 g/l Tricine (pH 7.4) with 50 μg/ml of bovine serum albumin [21]) at 37°C and incubated for increasing lengths of time with 36 pM ^{125}I-ANP in the absence (total binding) or presence (nonspecific binding) of 100 nM unlabeled human ANP. This concentration of ^{125}I-ANP was selected because it is similar to the physiologic concentration of ANP (3.3–23.3 pM) in the plasma [22] and has sufficient radioactivity to determine ^{125}I-ANP binding to the cells. After each time point, the cells were washed with BM at 4°C and incubated with an acid wash solution (0.2 M acetic acid, 0.5 M NaCl, pH 2.5) for 10 min at 4°C to remove the surface-bound ^{125}I-ANP [10]. Approx. 85% of the surface-bound ^{125}I-ANP was removed by the acid wash (data not shown). The acid wash solution containing the surface-bound ^{125}I-ANP was collected, and the cells were solubilized in 1 M NaOH to determine the internalized ^{125}I-ANP. The ^{125}I-ANP bound to the cells was determined by adding the amounts of surface-bound and intracellular ^{125}I-ANP. The specific binding of ^{125}I-ANP was determined by

subtracting the nonspecific binding from the total binding; the nonspecific binding accounted for less than 20% of the total binding.

Effect of temperature on ^{125}I-ANP degradation

CPA47 cells, cultured in 12-well plates, were washed with BM at 4°C or 37°C. To protect ^{125}I-ANP from nonspecific proteinases, a > 400000-fold excess of BSA (50 μg/ml by weight) to radiolabeled ANP (0.11 ng/ml) was present in the binding medium. For the 37°C experiment, cells, binding medium or medium conditioned by cells for 1 h at 37°C were incubated with 36 pM ^{125}I-ANP at 37°C for 1 h. For the 4°C experiment, cells, binding medium or medium conditioned by cells for 4 h at 4°C were incubated with 36 pM ^{125}I-ANP for 4 h at 4°C. After the incubations at different temperatures, the medium was collected, and frozen at −70°C until analyzed by HPLC.

Effect of EDTA on the degradation of ^{125}I-ANP by CPA47 cells and conditioned medium

CPA47 cells were cultured in 12-well plates until 90% confluent. All cells were washed twice with BM at 4°C, and then washed with either BM, BM with 2 mM EDTA, or BM with 5 mM EDTA at 4°C. The concentrations of EDTA were selected to chelate the 1.8 mM divalent cations present in the binding medium. CPA47 cells or medium conditioned by cells (CM) for 1 h at 4°C were incubated for 1 h at 4°C with 35–45 pM ^{125}I-ANP in 0.4 ml. After incubation, the medium was collected and centrifuged at 13000 × *g* for 2 min to remove any large debris, and was frozen at −70°C until assayed. ^{125}I-ANP degradation was determined by Sep-Pak fractionation.

Effect of hyperosmolarity on ^{125}I-ANP degradation by CPA47 cells

CPA47 cells were cultured in 12-well plates and grown to confluence. The cells were washed twice in BM with 2 mM EDTA at 37°C. Hyperosmolarity was induced by the addition of 0.2 M sucrose [23] into the binding medium. CPA47 cells were incubated with or without 0.2 M sucrose in 0.4 ml of BM with 2 mM EDTA at 37°C for 5 min. Radiolabeled ANP (75 pM) was then added to the cells, which were incubated for 20 min at 37°C. Increasing the ^{125}I-ANP concentration to 75 pM was necessary to increase the sensitivity of the assay as a result of the brief incubation period (20 min vs. 60 min). After the 20 min incubation, the medium was removed, centrifuged for 2 min at 13000 × *g* and a Sep-Pak assay was performed to quantitate ANP degradation.

HPLC analysis of ^{125}I-ANP degradation

ANP was analyzed by HPLC as previously described by Chen et al. [24]. Briefly, the medium was pooled, acidified with 0.4 M HCl, and adsorbed onto a C_{18} Sep-Pak cartridge (Waters, Milford, MA). ^{125}I-ANP was eluted from the Sep-Pak cartridge with 60% acetonitrile in 0.1% TFA and the samples were dried under nitrogen. The dried samples were dissolved in 0.1% acetic acid and applied to a μ-Bondapack C_{18} HPLC column (Waters, Milford, MA). The mobile phase was a linear gradient of 10 to 60% acetonitrile in 0.1% TFA with a flow rate of 1 ml/min for 60 min. The radioactivity in each fraction was determined by a Packard Auto-Gamma 500 counter. The percent degradation of ^{125}I-ANP was calculated by the following equation:

$$\%\ \text{degradation} = [\text{degraded } ^{125}\text{I-ANP}/(\text{intact} + \text{degraded } ^{125}\text{I-ANP})] \times 100$$

The intact ^{125}I-ANP eluted from the C_{18} HPLC column between 30 to 33 min. The radioactivity recovered in other fractions was considered degraded ^{125}I-ANP.

Sep-Pak analysis of ^{125}I-ANP degradation

^{125}I-ANP degradation was determined by Sep-Pak fractionation according to Morel et al. [25] with minor modifications. Briefly, samples were acidified with 0.4 M HCl and adsorbed onto C_{18} Sep-Pak cartridges. The degraded ^{125}I-ANP was eluted with 6 ml of 20% acetonitrile in 0.1% TFA. The remaining radioactivity on the Sep-Pak cartridge represented the intact ^{125}I-ANP. The percent degraded ^{125}I-ANP was calculated using the equation above for HPLC degradation. The percent degradation of ^{125}I-ANP as determined by HPLC was within 6% of the degradation determined by Sep-Pak fractionation (data not shown).

Results

Kinetics of ^{125}I-ANP binding to CPA47 cells at 37°C

^{125}I-ANP bound rapidly to CPA47 cells at 37°C. However, the binding peaked after 10 min and then decreased 76% after 60 min with little intracellular accumulation of ^{125}I-ANP (Fig. 1). The rapid decrease in ^{125}I-ANP binding to CPA47 cells was unexpected. The most likely explanation for the 76% decrease in specific ^{125}I-ANP binding to CPA47 cells is the degradation or modification of ^{125}I-ANP by the CPA47 cells.

^{125}I-ANP degradation by CPA47 cells

Degradation of ^{125}I-ANP by CPA47 cells at 4°C and 37°C. To establish that ^{125}I-ANP was being degraded by the CPA47 cells, ^{125}I-ANP in the medium that was incubated with cells was analyzed using HPLC. Medium samples were obtained after 1 h incubation at 37°C or after 4 h incubation at 4°C. Because receptor-mediated and fluid-phase endocytosis are minimal at 4°C [26],

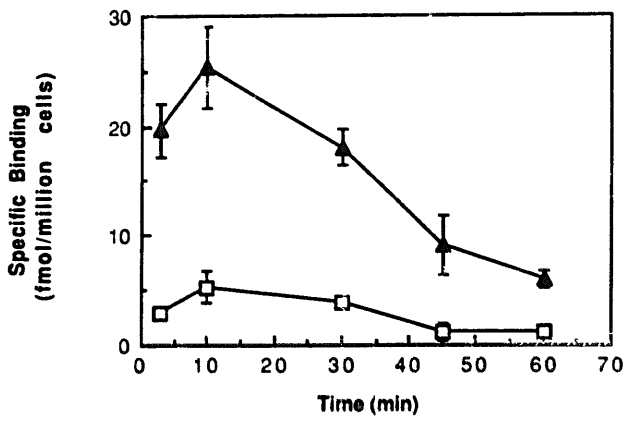

Fig. 1. Kinetics of ^{125}I-ANP binding to CPA47 cells at 37°C. Cells were incubated for different lengths of time with 36 pM ^{125}I-ANP in the absence and presence of 100 nM unlabeled human ANP and the surface-bound and intracellular ^{125}I-ANP was determined as described in Methods. The closed triangles represent the ^{125}I-ANP specifically bound to CPA47 cells and the open squares represent the internalized ^{125}I-ANP. The data represent the means and sample standard deviation (S.D.) of three independent experiments.

this temperature was selected to confirm that ^{125}I-ANP was degraded at or near the cell surface. These time points were selected for initial experiments because of the 76% loss in ^{125}I-ANP binding after 1 h incubation at 37°C, and because of the slowing of enzymatic activity at 4°C.

Intact radiolabeled ANP or radiolabeled ANP incubated in binding medium (BM) at 37°C for 1 h eluted between 30 and 33 min on a C_{18} HPLC column (Fig. 2). However, radiolabeled ANP was converted into fragments by CPA47 cells, and medium conditioned by cells (Fig. 2). The majority of these fragments were eluted between 15 and 20 min. These peaks possibly correspond to ^{125}I-Tyr and Arg-^{125}I-Tyr, based on previous observations using similar HPLC gradients [9,15,28].

HPLC analysis of radiolabeled ANP indicated that ^{125}I-ANP was 88% degraded by CPA47 cells after 60 min at 37°C (Table I). Furthermore, ^{125}I-ANP was degraded to the same extent by CPA47 cells after 4 h at 4°C (Table I). Controls verified that radiolabeled ANP was not significantly degraded by BM, BM from rinsed plastic or BM from serum coated plastic after 60 min at 37°C (< 7% ^{125}I-ANP degradation). However, medium conditioned by CPA47 cells at 37°C or 4°C that was incubated for 1 h at 37°C or 4 h at 4°C degraded 41% and 54% of the added ^{125}I-ANP, respectively (Table I). Thus, a proteolytic activity was released by CPA47 cells into the medium. However, the degradation of radiolabeled ANP in the conditioned medium did not account for all the degradation observed at 4°C for 4 h. Thus, these data suggest that a cell-surface proteolytic activity exists on CPA47 cells.

Effect of EDTA on ^{125}I-ANP degradation by CPA47 cells and conditioned medium. In order to verify the existence of the surface proteolytic activity, the proteolytic activity in the conditioned medium had to be selectively inhibited. A variety of protease inhibitors were tested to inhibit the proteolytic activity in the conditioned medium. EDTA, a divalent cation chelator, was found to be the most effective inhibitor of this proteolytic activity. The proteolytic activity in the CM was inhibited 84% and 72% by 2 mM or 5 mM EDTA, respectively (Table II). However, the degradation of ^{125}I-ANP by CPA47 cells was inhibited only 31% by BM with 2 mM EDTA or 39% by BM with 5 mM EDTA; moreover, at 4°C, degradation of ^{125}I-ANP by CPA47 cells, in the presence of either concentration of EDTA, was significantly greater ($P < 0.01$) than in conditioned medium.

Furthermore, the degradation of ^{125}I-ANP by the cells and conditioned medium was additive, suggesting that two different proteolytic activities were present. The calculated sum of ^{125}I-ANP degraded by cell-derived proteolytic activity (i.e., conditioned medium in BM) and ^{125}I-ANP degraded by cell-surface activity (i.e., cells incubated in BM with 2 mM EDTA) is 70% ± 11% per 10^5 cells ($n = 5$). The actual degradation of ^{125}I-ANP observed by cells incubated in BM (i.e., degradation of ANP by both the cell-surface and cell-derived proteolytic activities) was 59% ± 10% per 10^5 cells ($n = 5$). There was no statistical difference between the calculated and the observed degradation of ^{125}I-ANP ($P > 0.1$). Thus, CPA47 cells seem to have two distinct ^{125}I-ANP proteolytic activities: one at the cell-surface that does not require divalent cations, and one released into the medium that requires divalent cations.

An EDTA concentration of 2 mM EDTA was used in subsequent experiments because there was no statistically significant difference between the % degradation of ^{125}I-ANP by CM in 2 mM or 5 mM EDTA. In addition, cells adhered to the culture plate better in binding medium containing 2 mM EDTA than 5 mM EDTA.

Effect of hyperosmolarity on ^{125}I-ANP degradation by CPA47 cells at 37°C. Hyperosmolarity was used to inhibit receptor-mediated endocytosis at 37°C to assess the contribution of ^{125}I-ANP degradation by the cell-surface proteolytic activity. Hyperosmolarity has been demonstrated to inhibit receptor-mediated endocytosis in hepatocytes and in liver endothelial cells [23,27]. Hence, a cell-surface proteolytic activity was implied at 37°C when receptor-mediated endocytosis was inhibited by hyperosmolarity. The difference between cells treated in normal medium and cells treated in hyperosmotic medium indicated the fraction of ^{125}I-ANP degradation by receptor-mediated endocytosis.

When cells were incubated with BM containing 2

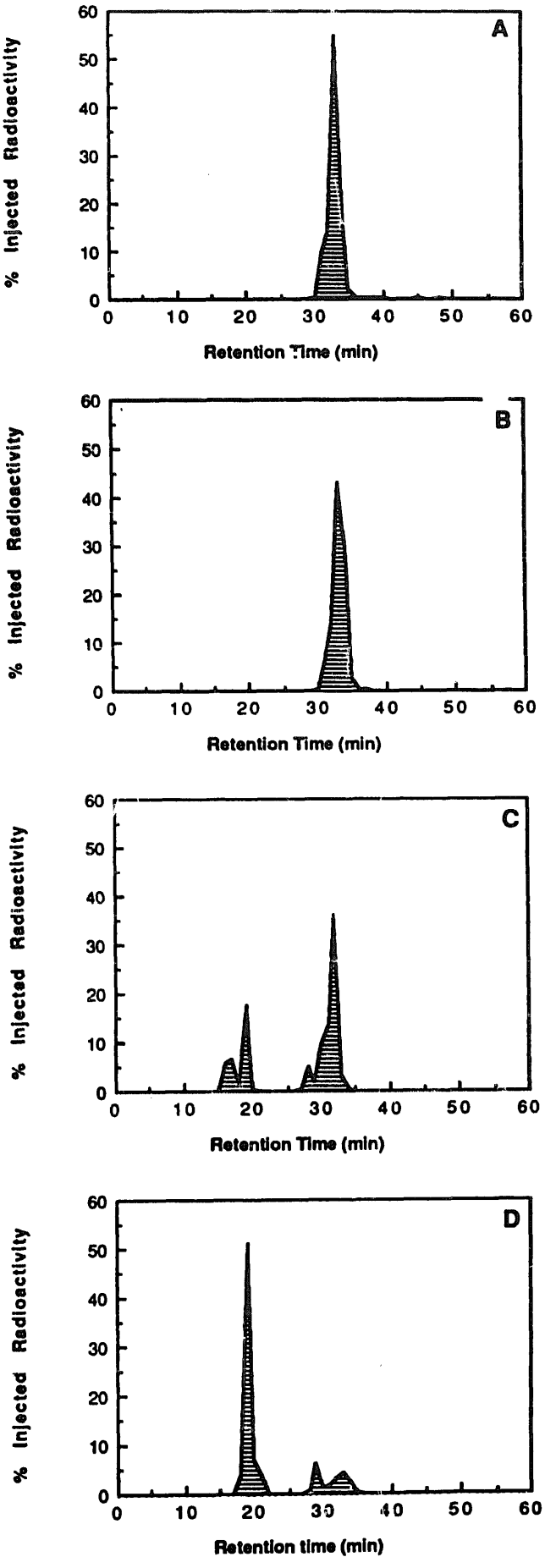

Fig. 2. ^{125}I-ANP degradation by CPA47 cells at 37°C. Cells, medium conditioned by cells for 1 h at 37°C, or BM alone were incubated with 36 pM ^{125}I-ANP at 37°C for 1 h, the medium was collected and frozen at −70°C until analyzed by HPLC as described in Methods. (Panel A) ^{125}I-ANP in BM and immediately frozen at −70°C. (Panel B) ^{125}I-ANP in BM incubated at 37°C for 1 h and then frozen. (Panel C) ^{125}I-ANP incubated with medium previously conditioned by CPA47 cells. (Panel D) ^{125}I-ANP incubated in BM with CPA47 cells.

TABLE I

Effect of temperature on the degradation of ^{125}I-ANP by CPA47 cells

The effect of temperature on the degradation of ^{125}I-ANP by cells, binding medium or medium conditioned by cells was performed as described in Methods. The degraded radiolabeled ANP was analyzed by HPLC as described in Methods. The data are presented as the means ± S.D. of two independent experiments.

Treatment	% degraded
^{125}I-ANP incubation at 37°C	
With cells	88 ± 1
Conditioned medium	41 ± 3
Without cells	5 ± 2
^{125}I-ANP incubation at 4°C	
With cells	88 ± 3
Conditioned medium	54 ± 8
Without cells	7 ± 1

TABLE II

Effect of EDTA on ^{125}I-ANP degradation by CPA47 cells and conditioned medium

CPA47 cells were washed twice with BM at 4°C, and washed with BM, BM with 2 mM EDTA or BM with 5 mM EDTA. CPA47 cells or medium conditioned by cells for 1 h at 4°C were incubated for 1 h at 4°C with 36 pM ^{125}I-ANP in 0.4 ml. After incubation, the medium was collected, centrifuged at 13000 × *g* for 2 min, and frozen at −70°C until assayed. ^{125}I-ANP degradation was was determined by Sep-Pak fractionation as described in Methods. The data represent the means ± S.D. of five independent experiments for cells with BM and BM with 2 mM EDTA, and three independent experiments with BM containing 5 mM EDTA.

Medium	^{125}I-ANP incubation	ANP degradation
BM	with cells	59 ± 10 [a]
	with CM	29 ± 6
BM + 2 mM EDTA	with cells	41 ± 6 [a]
	with CM	7 ± 4
BM + 5 mM EDTA	with cells	36 ± 10 [b]
	with CM	7 ± 5

[a] % degradation by cells is greater than conditioned medium ($P < 0.005$).

[b] % degradation by cells is greater than conditioned medium ($P < 0.01$).

mM EDTA for 20 min at 37°C, 28% ± 3% ($n = 3$) of radiolabeled ANP was degraded in the medium per 10^5 cells. When cells were incubated with medium

containing 0.2 M sucrose in the EDTA-containing BM, degraded ^{125}I-ANP recovered in the medium per 10^5 cells was 13% ± 1% ($n = 3$). Thus, receptor-mediated endocytosis and the cell-surface proteolytic activity are surmised to degrade 15% (i.e., 28%–13%) and 13% of the intact ^{125}I-ANP in the medium per 10^5 cells, respectively. Hence, the cell-surface proteolytic activity is responsible for almost half (13%/28%) of the ^{125}I-ANP degraded by the cells at 37°C in the presence of 2 mM EDTA.

Discussion

ANP plays a crucial role in salt and water homeostasis. The regulation of ANP plasma concentrations in vivo are poorly understood. The initial biological response to increased atrial pressure appears to be the release of ANP from the right atrium of the heart into the bloodstream [2]. However, ANP degradation modulates the time and extent of ANP's biological effects. When ANP is injected with thiorphan or phosphoramidon, which inhibit NEP 24.11, the biological effects of ANP are increased and prolonged compared with ANP injection alone [6]. Thus, degradation is important in the control of ANP's biologic activity.

Our study confirms that ^{125}I-ANP can be degraded by receptor-mediated endocytosis and by a proteolytic activity released from endothelial cells [15,18]. The degradation of ^{125}I-ANP by conditioned medium could be inhibited 84% by 2 mM EDTA, suggesting that the cell-derived factor in conditioned medium is a metalloproteinase. It is unlikely that the cell-derived factor from medium conditioned by CPA47 cells is secreted by the cells, since at 4°C receptor-mediated and fluid-phase endocytosis is inhibited and membrane fluidity is reduced [26]. Therefore, this factor is likely to be an enzyme present in the extracellular matrix or an enzyme present on the cell-surface that is released into the medium with increasing time. These data are similar to those reported by Johnson and co-workers [15] in which they observed ^{125}I-ANP degradation by conditioned medium in BAEC. However, the soluble proteolytic activity present in medium conditioned by CPA47 cells was 84% inhibited with 2 mM EDTA, while the enzyme present in medium conditioned by BAEC reported by Johnson and Foster [18] could be inhibited only approx. 35% by EDTA.

Our data also support the presence of a previously undescribed divalent cation-independent proteolytic activity (or activities) for ^{125}I-ANP on the surface of endothelial cells. Five millimolar EDTA chelated the 1.8 mM divalent cations present in the binding medium as well as the available divalent cations present on the cells. Significant ^{125}I-ANP degradation occurred when CPA47 cells were incubated at 4°C in the presence of 5 mM EDTA, suggesting that cell-surface proteolytic activity does not require divalent cations. Furthermore, the cell-surface divalent cation-independent activity appears to have carboxypeptidase-like activity since potato carboxypeptidase inhibitor could prevent > 90% of the ^{125}I-ANP degradation by CPA47 cells in the presence of 2 mM EDTA at 4°C (Frost, S.J. and Whitson, P.A., unpublished data). Therefore, the divalent cation-independent surface proteolytic activity on CPA47 cells is unlikely to result from NEP 24.11 because NEP 24.11 is a metalloproteinase with the ability to degrade ANP in the presence of potato carboxypeptidase inhibitor [11]. Thus, receptor-mediated endocytosis, a soluble proteolytic activity, and a divalent cation-independent proteolytic activity are responsible for the clearance and degradation of ^{125}I-ANP by endothelial cells.

One possible physiologic benefit of cell-surface ANP proteolysis on endothelial cells would be the rapid inactivation of ANP's biologic activity. Another benefit of a cell-surface ANP proteolytic activity on endothelial cells is the continued degradation of ANP if receptor-mediated endocytosis were to be inhibited. Interestingly, other biologically active peptides apparently are degraded by cell-surface peptidases on endothelial cells [19,20]. For example, dipeptidyl peptidase IV has been reported on the surface of endothelial cells and is able to modify biologically active peptides such as substance P, pancreatic polypeptide, and neuropeptides [19,20]. However, dipeptidyl peptidase IV could not be responsible for ^{125}I-ANP degradation, because ^{125}I-ANP does not possess the proline residue required for this enzyme's activity [19]. Another possible consequence of a cell-surface or cell-derived proteolytic activity is the generation of a modified ANP. Modifications to ANP could be physiologically relevant since some truncated analogues of ANP retain biological activity [29]. Whether the ANP fragments resulting from proteolysis by CPA47 cells prove to be biologically significant remains to be determined.

Although CPA47 cells appear to degrade ^{125}I-ANP at the cell surface, the biochemical mechanism for this process needs to be elucidated. Further biochemical characterization or purification of the cell-surface proteolytic activity from CPA47 cells will be necessary to clarify the role of surface ANP degradation in the metabolism of ANP in vitro and in vivo.

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