Characterization of natriuretic peptide production by adult heart atra

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Ogawa, Tsuneo, Marcelo Vatta, Benoit G. Bruneau, and Adolfo J. de Bold. Characterization of natriuretic peptide production by adult heart atra. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1977–H1986, 1999.—The cardiac polypeptide hormones atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) are synthesized and stored by atrial cardiocytes and share receptors and many biologic properties. Although some aspects of their synthesis and release are specific for each peptide, it is not clear whether they share intracellular sorting and secretory mechanisms.

In the present work we take advantage of a stable isolated rat atrial preparation that allows, for the first time, long-term study of synthesis, trafficking, targeting, and secretion of ANF and BNP by adult atrial muscle. Three model stimuli of secretion were used: increased intra-atrial pressure, endothelin-1 (ET-1), and phenylephrine (PE), representing mechanical, hormonal, and α1-adrenergic stimuli, respectively. To gain further insight into the secretory process under basal and agonist-induced secretion, we employed agents known to inhibit protein synthesis (cycloheximide) or to interfere with the vectorial transport of protein targeted for secretion (brefeldin A and monensin). All these agents induced significant changes in ANF and BNP release. Cycloheximide decreased natriuretic peptide secretion under basal and stimulated conditions. Brefeldin A dramatically increased basal as well as stimulated secretion of ANF and BNP. Monensin partially decreased basal ANF and BNP secretion and completely blocked stimulated secretion. None of these agents modified proteolytic processing as assessed by reverse-phase HPLC analysis. Double-label pulse-chase experiments using [3H]- and [14C]-lucine demonstrated that the secretory response to ET-1, in contrast to the response to muscle stretch, is based on peptide other than newly synthesized or relatively newly stored ANF. It is concluded that, in adult atrial cardiocytes, ANF and BNP are sorted to constitutive and regulated pathways in a manner that is substantially unique for atrial cardiocytes. In particular, it appears that basal and stimulated ANF and BNP secretion may have a large “constitutive-like” component, as previously defined in other endocrine systems. This type of secretion is based on the preferential release of hormone through vesicles arising from immature secretory granules. The capacity of the atria to release ANF and BNP in response to stimuli, therefore, may depend more on stimulation of the rate of formation of immature granules than on the amount of stored hormone.

atrial natriuretic factor; brain natriuretic peptide; endothelin-1; phenylephrine; stretch; synthesis; release

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ing that cycloheximide did not inhibit basal or stretch-stimulated ANF secretion (25), although using a double-label pulse-chase protocol, we reported that atrial stretch uses a pool of newly synthesized ANF (20). Similar findings have been described after investigations in which cultured adult rat atrial cells were used (16). From these investigations it is clear that release under basal and under stimulated conditions partly utilizes a pool of newly synthesized hormone that is promptly released, whereas a portion of the labeled hormone proceeds to a storage pool from which it is slowly released. This has been referred to as a “phasic” release (2).

An alternative secretory pathway, observed in the exocrine and endocrine pancreas, is based on the exocytosis of vesicles budding from immature granules (2, 3, 17). This type of secretion has been referred to as “constitutive-like.” It occurs under conditions in which regulated secretion is absent (3), it is independent from constitutive and regulated secretion, and it is cycloheximide insensitive (3, 17).

We recently established a novel model of adult rat right atria preparation that allows for experimentation periods of time extending to ≥9 h in an in vitro approach that closely duplicates physiological settings (6). In the present work we used this preparation together with three model stimuli previously recognized as relevant to the endocrine function of the heart: stretch stimulus through an increase in intra-atrial pressure, humoral stimulation with ET-1 as a stimulant, and neurohumoral stimulation with PE. We sought to gain insight into the processes of ANF and BNP release under basal and stimulated conditions using agents known to affect the synthesis and vectorial transport of secretory products. These included cycloheximide, a protein synthesis inhibitor at the ribosomal level; brefeldin A, a fungal metabolite used to inhibit protein transport between the endoplasmic reticulum (ER) and the TGN; and monensin, an ionophore that inhibits sorting and vesicle formation at the TGN (23, 24, 27). We report that regulated and constitutive pathways are evident in our preparation. These are specifically and uniquely affected by inhibition of protein synthesis or by disruption of the secretory processes by monensin and brefeldin A, whereas post-translational processing remains unaffected by all treatments or their combination. Furthermore, the bulk of the secretory response by atrial cardiocytes appears to be based on constitutive-like release.

**METHODS**

Isolation of right atria and perfusion protocols. The atrial preparation was as previously described (6). Briefly, hearts were removed from male Sprague-Dawley rats (300–350 g; Charles River Laboratories, Montreal, PQ, Canada) and placed in a supplemented Krebs-Ringer bicarbonate buffer (KRBB) solution. A PE-160 cannula (1.14 mm ID) was inserted into the inferior vena cava and exteriorized through the superior vena cava. The cannula had an opening midway facing the atrial chamber. The coronary sinus was ligated, the left auricle was removed, the lower five-sixths of the ventricles was removed, and a PE-10 cannula was inserted into the right atrium via the tricuspid valve.

The right atrium dissected in this manner was placed in a heated (30°C) organ chamber. The organ chamber contained 50 ml of KRBB that was replenished at a rate of 2.5 ml/min and gassed with 95% O2-5% CO2. The superior vena cava was attached to a cannula from which KRBB (preheated to 30°C and gassed with 95% O2-5% CO2) was infused at a rate of 3 ml/min. The outflow from the inferior vena cava was connected to a pressure transducer (Narco Bio-Systems, Houston, TX) that was connected to a physiograph (Narco Bio-Systems). The intra-atrial pressure was adjusted to 0.5 mmHg. The flow of KRBB was from the superior vena cava, into the right atrium, and out the tricuspid valve. The perfusion medium was collected for 5-min periods in siliconized glass tubes by use of a fraction collector (Retriever II, ISCO, Lincoln, NE).

The atria were allowed to equilibrate for 80 min, then perfusion medium was collected for 5 min of each 20-min period. A 50-µl aliquot was transferred to another tube for the ANF RIA. The remaining medium was kept for extraction for BNP RIA. Both tubes were kept at −20°C until use. At 30 min, cycloheximide (final concentration 10 µg/ml), brefeldin A (final concentration 7 × 10⁻⁶ M), or monensin (final concentration 5 × 10⁻⁶ M) was added to the KRBB until the end of the experiment. In each experiment, three kinds of stimulations were started at 120 min: stretch stimulation, ET-1 stimula-

Fig. 1. Basal atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP) release from isolated atria and effect of cycloheximide on secretion. Atria were perfused with vehicle (○) or cycloheximide (10 µg/ml) starting at 120 min and continuing to end of experiment (360 min; ●). irANF and irBNP, immunoreactive ANF and BNP. Values are means ± SE; n = 5 for ANF and n = 3 for BNP. *P < 0.05.
tion, and PE stimulation. 1) Stretch stimulation was accomplished by placing a solenoid-driven piston midway along the outflow cannula, which acted as a valve. The solenoid closing rate and duration were adjusted so that the intra-atrial pressure increased from 0.5 to 8 mmHg. 2) ET-1 was added to the KRBB at a final concentration of $1 \times 10^{-8}$ M. 3) PE was added to the KRBB at a final concentration of $5 \times 10^{-5}$ M. Unless otherwise stated, all stimulations were carried out for 240 min. Control atria were incubated in the same manner but without stimulation.

Double-label pulse-chase protocol. The atria, prepared as described above, were allowed to equilibrate for 80 min, then they were incubated in 50 ml of recirculated leucine-free KRBB containing $[1^4C]$leucine (Du Pont, Markham, ON, Canada; 315 mCi/mmol, final concentration 10 µCi/ml) for 180 min to uniformly label the storage pool (20). After this period of labeling, the atria were perfused with standard KRBB for 60 min of chase period, during which the perfusion medium was collected every 5 min. After this period the atria were incubated for 60 min in 50 ml of recirculated, leucine-free KRBB containing the second label, $[3^H]$leucine (Du Pont; 145 Ci/mmol, final concentration 50 µCi/ml) to label the more recently synthesized NP pool. The atria were then perfused with standard KRBB for 60 min during the second chase period with or without ET-1 (final concentration $1 \times 10^{-8}$ M), and the perfusion medium was collected every 5 min. A 50-µl aliquot was transferred to another tube for ANF RIA, and the remaining medium was kept for extraction for BNP RIA and immunoprecipitation. Both tubes were kept at −20°C until use. At the end of the experiment, the atria were flash frozen in liquid nitrogen and kept at −80°C until immunoprecipitation was performed. For this purpose, 15 ml of perfusate or atrial sample were extracted and freeze-dried as described below. The freeze-dried samples were resuspended in 1.5 ml of saline. One hundred microliters of a 1:10 dilution of nonspecific antibody were added to 100 µl of the resuspended sample and incubated at 4°C overnight. One hundred microliters of a mixture of goat anti-mouse IgG serum and polyethylene glycol (Peninsula, Belmont, CA) were then added, and the tubes were left at room temperature for 2 h. After centrifugation at 2,000 g for 45 min at 4°C, 100 µl of a 1:10 dilution of anti-ANF antibody were added to the supernatant and incubated at 4°C overnight. One hundred microliters of the goat anti-mouse IgG serum and polyethylene glycol mixture were then added, and the tubes were left at room temperature for 2 h. After centrifugation at 2,000 g for 45 min at 4°C, 1 ml of NCS tissue solubilizer (Amersham, Oakville, ON) was added to the pellet and incubated at 40°C overnight. Cytoscint (10 ml) was added to the sample, and the radioactivity was determined by liquid scintillation counting. $^3H$ and $^{14}C$ disintegrations per minute were computed with the use of external standard-based quench corrections.

Extraction of the perfusion media and tissue samples. For immunoprecipitation, the perfusion media samples were passed through Sep-Pak C18 cartridges (Millipore, Milford, MA) that were prewetted with 5 ml of 80% acetonitrile (ACN) in 0.1% trifluoroacetic acid (TFA) and washed with 10 ml of 0.1% TFA. The cartridges with the adsorbed peptides were washed with 20 ml of 0.1% TFA and then eluted with 3 ml of 80% ACN in 0.1% TFA. The eluates were freeze-dried and resuspended in RIA buffer. ANF and BNP RIAs in tissue were carried out in extracts obtained by homogenizing the atria in 3 ml of an extracting mixture consisting of 0.1 N HCl, 1 M acetic acid, and 1% NaCl and centrifuged at 10,000 g for 30 min. The supernatant was then passed through Sep-Pak C18 cartridges that were prewetted with 5 ml of 80% ACN in 0.1% TFA. The cartridges with the adsorbed peptides were then washed with 10 ml of 0.1% TFA, and the eluates were freeze-dried and resuspended in RIA buffer. ANF and BNP RIAs were performed on the extracts and tissue samples, respectively, using commercially available kits (Amersham, Oakville, ON) according to the manufacturer’s instructions.

Fig. 2. Effect of cycloheximide on stimulated ANF and BNP release. Secretagogues (stretch [intra-atrial pressure change from 0.5 to 8 mmHg], endothelin-1 [ET-1, $1 \times 10^{-8}$ M], and phenylephrine [PE, $5 \times 10^{-5}$ M]) were started at 120 min with (c) or without (o) pretreatment with cycloheximide (10 µg/ml). Pretreatment with cycloheximide was started at 30 min and continued to end of experiment (360 min). For stretch experiments only, a longer pretreatment with cycloheximide was started at 30 min, and stretch was carried out at 210 min (c). Values are means ± SE; n = 5 for ANF and n = 3 for BNP. *P < 0.05; **P < 0.01 for stimulated secretion vs. basal values in Fig. 1. Cycloheximide treatment lowered nonsignificantly stimulated secretion.
min at 4°C. The supernatants were then extracted using Sep-Pak C_{18} cartridges, as described above for the perfusion medium.

Protein synthesis inhibition. For these investigations, the perfusions were carried out with KRBB without phenylalanine. The atria were allowed to equilibrate for 80 min, then they were treated with different doses of cycloheximide (final concentrations 0, 0.1, 1, and 10 µg/ml). Thirty minutes after the cycloheximide treatment was started, [14C]phenylalanine (Amersham; >450 mCi/mmol, final concentration 0.1 µCi/ml) was added to the KRBB and continued for 60 min, then the atria were washed with standard KRBB for 10 min. At the end of the experiment the atria were flash frozen in liquid nitrogen and kept at −80°C until use. For the determination of [14C]phenylalanine incorporation, the atria were homogenized in 10 ml of 10% TCA containing 10 mg/ml of phenylalanine and centrifuged at 1,600 g for 15 min at 4°C. The pellet was then homogenized and centrifuged under the same conditions. The resulting pellet was weighed and then incubated in 1 ml of NCS tissue solubilizer (Amersham) for 24 h at 40°C. Cytoscin (10 ml) was added to the solubilized pellet, and the incorporated radioactivity was determined by liquid scintillation counting.

Reverse-phase HPLC. Reverse-phase HPLC (RP-HPLC) analysis was performed as previously described (9) on a C_{18} column (0.78 × 30 cm; Vydac, Hesperia, CA) by use of a linear gradient of ACN from 15% to 55% in 0.1% TFA at a flow rate of 1.5 ml/min. Three-milliliter fractions were collected and assayed for ANF and BNP content.

ANF and BNP RIAs. RIA was performed using the double-antibody method, as previously described (29). Antiseras against rat ANF-(99—126) and rat BNP-(64—95) (Peninsula Laboratories) were used. Cross-reactivity of ANF and BNP antisera with BNP and ANF peptides, respectively, was <0.01%.

Statistical analysis. Values are means ± SE. To determine pairwise statistical differences, Student’s t-tests were performed. For multiple-groups comparison, one-way ANOVA and Bonferroni’s post hoc analysis were carried out. P < 0.05 was considered significant.

RESULTS

Effect of secretagogues on NP secretion. The kinetics of basal ANF and BNP secretion and the effect of three different stimuli of secretion (stretch, ET-1, and PE) are shown in Figs. 1 and 2. Total secretion over the 240-min test period is shown in Fig. 8. Atrial stretch induced an immediate increase in ANF and BNP secretion that peaked and started to decrease within 20 min to reach basal levels by 200 min. In contrast, ET-1 and PE induced a gradual increase in secretion that reached a plateau by 40 min and lasted to 280 min, when it started to decrease, although it did not return to basal levels by the end of the experiment (360 min). Stimulated BNP secretion by ET-1 or PE increased gradually, as did ANF, but remained distinctively above basal levels by the end of the observation period.

Effect of cycloheximide on NP secretion. Pilot experiments designed to optimize inhibition of protein synthesis by cycloheximide at 0.1, 1, and 10 µg/ml showed that protein synthesis was inhibited by 15, 85, and 99%, respectively (data not shown). The latter dose of cycloheximide significantly decreased total ANF and BNP release under basal conditions (Figs. 1 and 8) and stretch stimulation (Figs. 2 and 8). Cycloheximide tended to decrease ET-1-stimulated release and induced a slight increase in PE-stimulated release. Neither of these two changes, however, was statistically significant. To eliminate the possibility that cycloheximide treatment for 90 min was not sufficient for protein synthesis inhibition, another series of experiments in which stretch was started after 180 min of cycloheximide pretreatment was carried out. This longer pretreatment did not affect stretch response (data not shown).

Effect of brefeldin A on NP secretion. To assess the effect of blocking constitutive secretion and the formation of secretory granules on ANF and BNP secretion (24, 27), brefeldin A was introduced in the perfusates under basal and stimulated perfusion conditions. Brefeldin A induced an increase in basal ANF and BNP release starting 30 min after administration (Figs. 3 and 8). To determine whether the elevation in NP secretion by brefeldin A was due to cell necrosis, the reversibility of the effect was determined by discontinuing treatment at 160 min. At 200 min, NP secretion started to decrease; it returned to the basal level at 240 min (Fig. 3). Next, to investigate whether the increase in NP secretion by brefeldin A is dependent on newly synthesized ANF and/or BNP, the effects of cycloheximide pretreatment were carried out. This longer pretreatment was not sufficient for protein synthesis inhibition, another series of experiments in which stretch was started after 180 min of cycloheximide pretreatment was carried out. This longer pretreatment did not affect stretch response (data not shown).
synthesized hormone, the brefeldin A treatment was combined with cycloheximide. There was no significant difference in NP secretion between brefeldin A alone and brefeldin A plus cycloheximide (data not shown). Brefeldin A treatment dramatically increased stimulated ANF as well as BNP release. The effects were approximately additive (Figs. 4 and 8).

Effect of monensin on NP secretion. To determine the effect on NP release by inhibition of secretory vesicle formation without directly affecting protein synthesis (23), monensin was added to the perfusate under basal conditions or during stimulation by each of the three model stimuli. Monensin drastically decreased NP secretion within 30 min under all conditions tested and maintained the depressed level of secretion until the end of the experiment. To determine whether the monensin-insensitive component of basal secretion was dependent on newly synthesized protein, atria pretreated with cycloheximide were further treated with monensin. Unexpectedly, NP secretion after this combined treatment resulted in increased secretion compared with cycloheximide alone (Figs. 5–8).

RP-HPLC analysis. RP-HPLC analysis of perfusates from brefeldin A-treated atria showed that most of the ANF and BNP eluted in the position of ANF-(99–126) and BNP-(64–95), respectively. Thus the elution profile of immunoreactive ANF and BNP corresponded to the circulating form in vivo. This shows that nonspecific leakage did not occur with prolonged in vitro perfusion given that ANF propeptide (the predominant form of ANF in tissue) was not found in the perfusate (Fig. 9). Similar results were obtained with perfusates from control atria and after treatment with monensin, stretch, ET-1, and PE and the combinations described above (data not shown).

Double-label pulse-chase protocol. We previously demonstrated that stretch-induced ANF release partly uses a pool of newly synthesized peptide (20). In the present investigations we used ET-1 stimulation to determine whether the difference in release kinetics observed between the two stimuli is reflected in the utilization of different pools of NPs as visualized by the pulse-chase protocol. In this protocol, $[^{3}H]NP$ represents the NP synthesized $<1$ h before the start of ET-1 and is referred to as newly synthesized NP. $[^{14}C]NP$ represents NP synthesized 2–5 h before the start of ET-1 and represents the NP stored during this period. Thus nonlabeled NP is that synthesized $>5$ h before the start of ET-1 and represents the long-term storage of NP. ET-1-stimulated ANF secretion gradually and significantly increases ANF secretion into the medium (Fig. 10). The specific activity of $[^{14}C]ANF$, after it attains comparatively high values at the beginning of the chase, remained constant thereafter, including the

![Fig. 4](http://ajpheart.physiology.org/) Effect of brefeldin A on stimulated ANF and BNP release from isolated atria. Secretagogues [stretch (intra-atrial pressure change from 0.5 to 8 mmHg), ET-1 ($1 \times 10^{-7}$ M), and PE ($5 \times 10^{-5}$ M)] were started at 120 min with (●) or without (○) pretreatment with brefeldin A ($7 \times 10^{-6}$ M) and continued to the end of experiment (360 min). Control perfusions with brefeldin A alone are also shown (□). Values are means ± SE; n = 5 for ANF and n = 3 for BNP. *P < 0.05; **P < 0.01, secretagogues + brefeldin A vs. secretagogues alone. †P < 0.05; ‡P < 0.01, secretagogues + brefeldin A vs. brefeldin A alone.
period of ET-1 stimulation, during which the specific activities of the released ANF did not differ from that found in the basal state. A similar pattern was observed for \([3H]ANF\), although the specific activity during ET-1 treatment had a tendency to decrease compared with the basal group, indicating that the release of this newly synthesized ANF had been diluted by older, unlabeled ANF. No differences were found between the control and endothelin groups in the specific activities of \([14C]ANF\) or \([3H]ANF\) in tissue (data not shown). The lower amount of immunoprecipitable BNP did not allow for a reliable determination of specific activity of BNP in the immunoprecipitates.

**DISCUSSION**

Secretory function and morphology of atrial cardiocytes fulfill the general definition of regulated secretion, but the details of ANF and BNP storage and release defined through in vivo and in vitro studies show distinctive features for these hormones (8). In the present study we show that ANF and BNP are sorted to constitutive and regulated secretory pathways and are secreted in a manner that appears unique to atrial cardiocytes.

Cycloheximide treatment of unstimulated atria resulted in a significant decrease in ANF and BNP secretion, thus showing that the basal release of these NPs is partly dependent on newly synthesized hormone, which suggests constitutive release, and partly dependent on stored hormone. At variance with these findings, Page et al. (25) reported that cycloheximide (100 µg/ml), in the presence of saxitoxin (used to inhibit spontaneous action potentials) and ryanodine (used to block ryanodine-sensitive \(Ca^{2+}\) release from the sarcoplasmic reticulum as well as to inhibit spontaneous contractions) did not affect basal ANF secretion from a perfused rat atrium preparation and concluded that there is no constitutive pathway in NP secretion. In agreement with the present studies, however, Doubell and Thibault (12) reported that cycloheximide (10⁻⁶ M) partially downregulated ANF secretion from cultured neonatal atrial cells. Iida and Shibata (16), using cultured atrial cardiocytes from adult rats, concluded that ~40% of newly synthesized, radiolabeled ANF was partially secreted under unstimulated conditions and the remainder of the newly synthesized ANF was destined for storage or later slow release.

In the present investigation we observed that cycloheximide had a tendency to decrease NP secretion under stimulatory conditions, suggesting that the constitutive pathway may also be inhibited under these conditions, although the bulk of the response was still...
evident. This is in partial agreement with previous studies that showed no effect of protein synthesis inhibition on stimulated ANF secretion (12, 25). Therefore, most of the response to stimulation is based on previously synthesized, stored hormone, and “newly synthesized” ANF is but a minor component of this response. Using the isolated rat atria and a double-isotope-labeling pulse-chase protocol, we previously found (20) that stretch stimulated newly synthesized ANF release and that there is no change in the specific activity of the older, stored ANF pool that had been labeled 2–5 h before stretch. In light of the present studies, i.e., the fact that cycloheximide does not significantly affect stretch-regulated ANF release, it appears that the bulk of ANF released by stretch is derived from a pool synthesized 5 h before stretch.

Brefeldin A, a fungal metabolite, prevents the assembly of non-clathrin-coated vesicles from the Golgi cisternae; thus this compound is a very effective blocker of constitutive secretion and of the formation of secretory granules (24, 27). In the rat pancreas, brefeldin A blocks ER to Golgi transport but does not affect later stages along the secretory pathway, including intra-Golgi transport, exit from the Golgi complex, formation and concentration of secretory granules, and exocytosis (14). We found here that basal and stimulated NP secretion was enhanced by brefeldin A. This elevation in NP secretion was not due to cell damage, since the effect of brefeldin A was readily reversible, and full processing to the mature secreted forms of ANF and BNP was maintained. The enhancement of NP release by brefeldin A is consistent with previous investigations in which ANF secretion was reported to be enhanced by brefeldin A in the perfused rat atrium (25) and in cultured neonatal rat cardiocytes (11). A possibility underlying these observations is that brefeldin A might block the transport of unknown proteins that modulate vesicular formation or storage vesicle traffic at the TGN or later levels; however, if this were the case, it may be expected that cycloheximide might also enhance NP secretion. Lippincott-Schwartz et al. (19) proposed that brefeldin A divides the secretory pathway into two functionally distinct, noncommunicating “homotypic systems”: the proximal system is made up of a fusion of ER and Golgi stacks, and the distal system consists of the TGN, endosomes, and plasma membrane elements collapsed together. The transport across the two homotypic systems is blocked, whereas traffic within the homotypic system continues in brefeldin A-treated cells. Morphologically, each homotypic compartment is seen to consist of fused elements (32). This suggests that, in atrial cardiocytes, NP storage vesicles may be homotypic to the distal system (TGN, endosomes, and plasma membrane), allowing for a facilitated access to the plasma membrane, thus resulting in an increased secretion rate, even though newly synthesized NP transport is blocked. This hypothesis seems to be supported by the fact that cycloheximide did not...
affect the increased NP secretion induced by brefeldin A. These findings suggest that all elements required for regulated secretion and its characteristic secretagogue-induced kinetics lie in the distal homotypic compartment defined by brefeldin A.

Monensin is an ionophore that induces the movement of \( \text{Na}^+ \) into cellular compartments in the central vacuolar system by \( \text{Na}^+/	ext{H}^+ \) exchange, increasing the pH and thus impairing protein sorting and transport in the TGN and inhibiting vesicle formation without directly affecting protein synthesis (23). In our preparation, monensin significantly decreased basal NP secretion but did not completely stop secretion. This effect is consistent with our earlier observations with a different isolated atrial preparation (10), and it has been observed in other secretory processes (28) and for the release of ANF from cultured adult rat atrial cardiocytes (15). Because we found that cycloheximide reduced the level of basal secretion, we combined this drug with monensin to determine whether an additional inhibitory effect on basal secretion would become apparent. Unexpectedly, NP secretion increased after these drugs were administered in combination, thus suggesting that the partial inhibition of NP release by monensin is not based on inhibition of synthesis. It is conceivable that cycloheximide inhibits the synthesis of a protein that negatively modulates ANF secretion at a site in the regulated component of basal NP release. In addition to its effect on basal ANF and BNP release, monensin also had a dramatic effect on stimu-
lated NP secretion, virtually obliterating the expected stimulatory effects of stretch, ET-1, and PE. Different results have been obtained in various investigations dealing with the effect of monensin on regulated release. Monensin blocked glucose-stimulated insulin release (13) and lipopolysaccharide-stimulated secretion of tumor necrosis factor (18). In contrast, monensin did not significantly affect corticotropin-releasing factor-stimulated ACTH release from the anterior pituitary (30) or mucin secretion in colonic tumor cells (22). Nevertheless, the fact that monensin was able to dramatically decrease agonist-induced NP secretion as well as a significant component of basal release, together with the fact that cardiocytes preferentially release newly synthesized hormone in a manner that does not entirely rely on protein synthesis, suggests that a constitutive-like release is the main release mechanism operating in atrial cardiocytes. This type of secretion has been characterized in the exocrine and endocrine pancreas, where it has been ascribed to the exocytosis of vesicles budding from immature granules (2, 3, 17). The term constitutive-like suggests that it continues under conditions in which regulated secretion is absent, is independent of constitutive and regulated secretion, and is cycloheximide insensitive (3, 17). Because this type of secretion depends on immature granules, it could be expected that it might be particularly susceptible to the increase in pH brought about by monensin treatment, as found in the present work. Together, these findings suggest that the capacity of the atria to increase ANF and BNP release in response to stimuli may depend more on stimulation of the formation of immature granules than on the amount of stored hormone.

The release kinetics for ANF and BNP observed in the present studies were very similar for both hormones. This is in contrast to previous observations in vivo that suggest a discoordinate regulation of hormone production. In previous investigations (33), we found that in rats treated for 1 wk with deoxycorticosterone acetate-salt, plasma ANF increased and left atrial ANF content decreased while plasma BNP and left atrial BNP content did not change. Continued deoxycorticosterone acetate-salt administration led to an increase in atrial BNP content, but the isolated granules showed a decrease in content, suggesting that the increase in BNP stores takes place in a cell compartment other than mature atrial granules. From the results obtained in the present investigation, we conclude that intracellular trafficking and secretory mechanisms responsible for basal and acute stimulated ANF secretion are likely to be shared by BNP, whereas long-term regulation of NP production may partly rely on mechanisms that are selective for each peptide.
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