Effect of Adrenergic and Muscarinic Cholinergic Agonists on Atrial Natriuretic Peptide Secretion by Isolated Rat Atria

Potential Role of the Autonomic Nervous System in Modulating Atrial Natriuretic Peptide Secretion

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Abstract

Stretching of the atrial wall is a known stimulant for atrial natriuretic peptide (ANP) secretion. Little is known about other factors that may influence ANP secretion. We examined the effects of the neurotransmitters of the autonomic nervous system on ANP secretion from isolated rat left atria. Superfusion with 10 μM norepinephrine produced a biphasic rise in ANP secretion with a peak response 2.5-fold above baseline secretion. To determine whether the response to norepinephrine primarily reflected α- or β-adrenergic receptor stimulation, atria were superfused with 0.1 μM isoproterenol or 10 μM phenylephrine and 1 μM propranolol. ANP secretion in response to isoproterenol was biphasic, similar to the response to norepinephrine. Phenylephrine evoked a monophasic ANP secretory response, which was delayed in onset relative to that of isoproterenol or norepinephrine. Superfusion with 10 μM methacholine alone had no effect on ANP secretion, but rapidly attenuated norepinephrine-stimulated secretion by 67%. From these observations we conclude: (*a*) Both α- and β-adrenergic agonists directly and distinctively stimulate ANP secretion; (*b*) Norepinephrine stimulates ANP secretion by both α- and β-adrenergic mechanisms, however the secretory response pattern of norepinephrine reflects a predominence of β-adrenergic activity; (*c*) Under basal conditions, methacholine does not influence ANP secretion; and (*d*) Methacholine inhibits norepinephrine-stimulated ANP secretion. Thus, in vivo, activation of the sympathetic nervous system may enhance ANP secretion, whereas a rise in parasympathetic tone may lower ANP secretion.

Introduction

Atrial natriuretic peptide (ANP),[1] a hormone secreted by the atria of the heart (1, 2), is believed to play an important physiological role in protecting the organism from volume overload due to its potent natriuretic and diuretic properties (3–6). However, little is known about factors that may influence ANP secretion. Mechanical distension or stretching of the atrial wall may be the primary physiological stimulus for ANP secretion (1, 7–9); however, ANP secretion may be modulated by other factors. Since atrial tissue is richly innervated by both sympathetic and parasympathetic nerve fibers, it is possible that the neurotransmitters norepinephrine or acetylcholine may influence ANP secretion. In this study, we examined the effects of adrenergic and muscarinic cholinergic agonists on ANP secretion by isolated rat left atria paced and superfused in vitro.

Methods

Female Sprague-Dawley rats weighing 200–225 g on an ad lib. sodium diet were killed by decapitation. Hearts were quickly removed and allowed to beat for 1–2 min in medium 199 with modified Earle's salts (KCl, 4.0 mM) gassed with 95% O_2/5% CO_2 to remove blood. Left atria were quickly removed, mounted, and superfused as previously described (10). Resting tension was initially set at 1.25 g and was not adjusted further. Atria were electrically paced at a rate of 1 Hz for 30 min and then at 2 Hz, except in the isoproterenol experiments where atria were paced at 3 Hz. After the pacing frequency adjustment, the atria were allowed to stabilize for 55 min. Thereafter, samples were collected at 2.5 min intervals. The following concentrations of agonists were used in these studies: 10 μM norepinephrine, 10 μM methacholine, 0.1 μM isoproterenol, and 10 μM phenylephrine. These concentrations of agonists were chosen since they produce a maximal change in developed tension. 100 μM ascorbic acid was added to the medium 199 for all experiments to decrease oxidation of adrenergic agonists.

ANP secretion was quantitated by RIA as previously described (10) with the following changes. Rat α-ANP was labeled with ^{125}I by the chloramine T method. Purification of the labeled hormone was achieved by reverse-phase HPLC using a Bondapak C_{18} column and a linear gradient from acetonitrile/water (1:12) to 100% acetonitrile. ^{125}I-ANP eluted at 65% acetonitrile.

The results are expressed as a percent of basal ANP secretion. Basal ANP secretion was defined as the mean of seven samples collected over 15 min immediately before the introduction of adrenergic or muscarinic cholinergic agonists.

Results

Representative tracings, illustrating the contractile responses of atria exposed to adrenergic and muscarinic cholinergic agonists, are presented in Fig. 1. Developed tension rose in response to the adrenergic agonists norepinephrine, isoproterenol, and phenylephrine and fell in response to the muscarinic cholinergic agonist methacholine. The contractile responses of atria exposed to these agents are summarized in Table I.

To examine the possibility that sympathetic or parasympa-

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1. *Abbreviations used in this paper:* ANP, atrial natriuretic peptide; ANP-IR, ANP-immunoreactive.

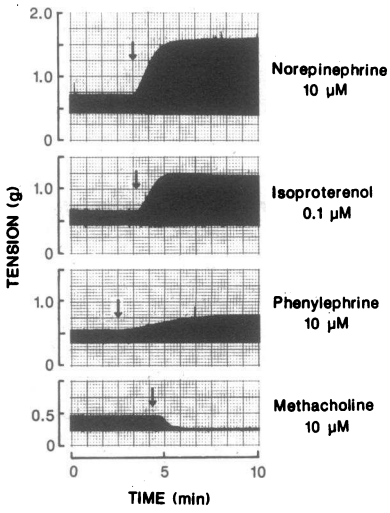

Figure 1. Tracing of atrial contractility. Representative tracings by paced rat left atria exposed to an adrenergic or muscarinic cholinergic agonist are presented. The arrows indicate the point in time where the agonist were introduced. Studies with phenylephine were performed in the presence of 1 μM propranolol.

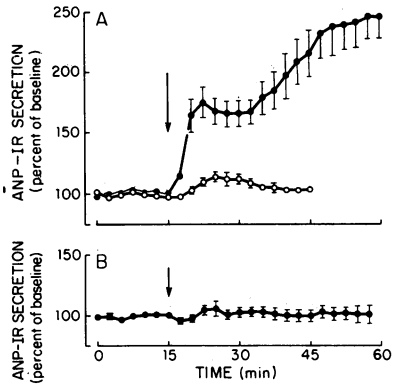

Figure 2. Effect of norepinephrine and methacholine on ANP-IR secretion by rat left atria paced at 2 Hz. (*A*) Atria were continuously superfused with 10 μM norepinephrine beginning at 15 min (*arrow*) in the absence (●, $n = 9$ atria) or presence (○, $n = 4$ atria) of 10 μM phentolamine and 5 μM propranolol. Error bars are SE and are occasionally contained within the symbols. Basal ANP-IR secretion was 236±19 pg/ml (mean±SE). (*B*) Methacholine 10 μM was continuously superfused beginning at 15 min ($n = 9$ atria). Basal ANP-IR secretion was 154±13 pg/ml.

thetic neurotransmitter release may influence ANP secretion, paced rat left atria were superfused with norepinephrine or methacholine, a more slowly hydrolyzed analogue of acetylcholine. Continuous superfusion with 10 μM norepinephrine for 45 min resulted in a biphasic ANP-immunoreactive (ANP-IR) secretory response (Fig. 2 *A*). The ANP-IR secretory response to norepinephrine was inhibited by simultaneous superfusion with 10 μM phentolamine and 5 μM propanolol (Fig. 2 *A*). ANP-IR secretion was not affected by superfusion with 10 μM methacholine (Fig. 2 *B*) in spite of a marked fall in developed tension (Table I).

Since norepinephrine possesses both α- and β-adrenergic agonist activity, experiments were designed to determine whether the ANP-IR secretory response to norepinephrine reflected an α- or β-adrenergic effect. Continuous superfusion with the β-adrenergic agonist isoproterenol (0.1 μM) resulted in a biphasic ANP secretory response (Fig. 3 *A*) similar to the secretory response elicited for norepinephrine (Fig. 2 *A*). The stimulatory effect of isoproterenol was inhibited by simultaneous superfusion with 1 μM propranolol (Fig. 3 *A*). The ANP-IR secretory response to α-adrenergic stimulation was examined by superfusing atria with 10 μM phenylephrine and 1 μM propranolol, since phenylephrine possesses a small amount of β-adrenergic agonist activity. Continuous superfusion with phenylephrine and propranolol resulted in a monophasic rise in ANP-IR secretion that was inhibited by simultaneous superfusion with 10 μM phentolamine (Fig. 3 *B*). The ANP-IR secretory response to phenylephrine was distinct from that of norepinephrine and isoproterenol, which were similar (Fig. 4). The secretory response to phenylephrine was less rapid in onset and did not produce the biphasic response typified by norepinephrine or isoproterenol stimulation. Thus, the pattern of the ANP secretory response to norepinephrine appeared to be similar to the β-adrenergic agonist isoproterenol.

These observations raised the question whether the secretory response to norepinephrine was exclusively a β-adrenergic

Table I. Contractile Responses of Electrically Paced Rat Left Atrial In Vitro to Adrenergic and Muscarinic Cholinergic Agonists

			Basal*	Experimental‡
	μM		g	g
Norepinephrine	0.25	RT§	0.56±0.04	0.54±0.04
($n = 5$)		DT‖	0.34±0.06	0.71±0.14¶
Norepinephrine	10	RT	0.24±0.03	0.19±0.03¶
($n = 9$)		DT	0.25±0.02	0.99±0.08¶
Isoproterenol	0.1	RT	0.31±0.04	0.28±0.03¶
($n = 6$)		DT	0.40±0.11	0.94±0.15¶
Phenylephrine	10	RT	0.25±0.02	NC**
($n = 6$)		DT	0.23±0.02	0.47±0.03¶
Methacholine	10	RT	0.29±0.04	NC
($n = 9$)		DT	0.25±0.02	0.06±0.01¶

* Basal measurements taken before superfusion with the test agent.
‡ Experimental measurements taken during superfusion with the test agent.
§ RT, Resting tension, mean±SE.
‖ DT, Developed tension (peak tension minus resting tension).
¶ $P < 0.02$ compared with basal measurements by paired t test.
** NC, No change.

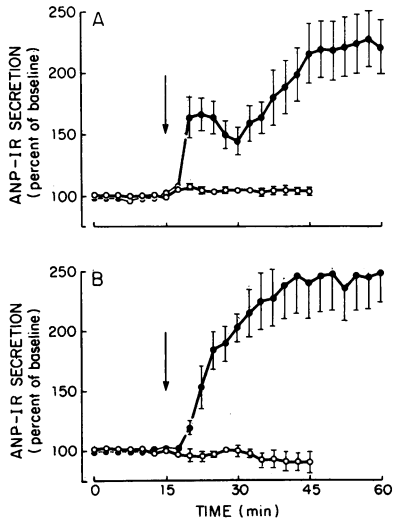

Figure 3. Effect of isoproterenol and phenylephrine on ANP-IR secretion by paced rat left atria. (*A*) Rat left atria paced at 3 Hz were continuously superfused with 0.1 μM isoproterenol in the absence (●, $n = 6$ atria) or presence (○, $n = 6$ atria) of 1 μM propranolol beginning at 15 min (*arrow*). Basal ANP-IR secretion was 198±25 pg/ml. (*B*) Rat left atria paced at 2 Hz were continuously superfused with 10 μM phenylephrine and 1 μM propranolol in the absence (●, $n = 6$ atria) or presence (○, $n = 3$ atria) of 10 μM phentolamine beginning at 15 min. Basal ANP-IR secretion was 158±24 pg/ml.

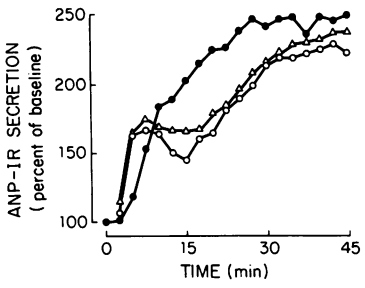

Figure 4. Comparison of ANP-IR secretion in response to norepinephrine (△), isoproterenol (○), and phenylephrine (●). The data are from Figs. 2 and 3. Error bars have been omitted for clarity. At 5 min, the secretory response to phenylephrine was significantly less than the responses to isoproterenol ($P < 0.014$) or to norepinephrine ($P < 0.016$) as determined by t test.

response or whether the response reflected a predominance of β-adrenergic activity. To answer this question, 10 μM norepinephrine was superfused separately with 10 μM phentolamine or 5 μM propranolol. Norepinephrine superfused in the presence of phentolamine resulted in a biphasic ANP secretory response (Fig. 5 *A*) similar to that noted for norepinephrine alone (Fig. 2 *A*) or isoproterenol (Fig. 3 *A*). A response to norepinephrine superfused with propranolol was also present (Fig. 5 *B*). The pattern of this response was similar to that seen for phenylephrine (Fig. 3 *B*). Thus, both the α- and β-adrenergic agonist properties of norepinephrine are capable of stimulating ANP secretion. Therefore, it appears that the ANP secretory response to norepinephrine reflects a predominance of β-adrenergic activity over that of an α-adrenergic effect.

In light of the observation that an α-adrenergic secretory response could be elicited by superfusion with norepinephrine and propranolol, the predominance of the β-adrenergic secretory pattern of norepinephrine was examined at a lower concentration of norepinephrine (0.25 μM). This concentration of norepinephrine resulted in a half-maximal increase in developed tension, whereas the 10-μM dose gave a maximal rise in developed tension. Superfusion with 0.25 μM norepinephrine continued to give a biphasic ANP secretory response pattern (Fig. 6). Thus, the predominance of the β-adrenergic secretory pattern of norepinephrine persists even at a lower concentration of norepinephrine.

Next to be examined was the possibility of an interaction between sympathetic and parasympathetic neurotransmitters on ANP secretion. Atria were initially superfused with 10 μM norepinephrine followed by the addition of 10 μM methacholine (Fig. 7). The results in this figure are expressed as the net percent change from baseline in ANP-IR secretion with the response at 45 or 47.5 min defined as 100%. Continuous superfusion with norepinephrine again resulted in a biphasic ANP-IR secretory response. The curve is less well defined in this experiment, since samples were collected every 5 min rather than every 2.5 min. Addition of methacholine continuously superfused from 45 to 75 min resulted in a dramatic fall in ANP-IR secretion to a nadir of 33±7% of the maximal response. Continuous superfusion with norepinephrine alone or addition of 10 μM methacholine in the presence of 10 μM atropine rendered ANP-IR secretion stable until the experiment was terminated. In these experiments, developed tension rose from 0.20±0.03 to 0.77±0.14 g with the addition of norepinephrine. Methacholine lowered developed tension to 0.19±0.05 g. Thus, methacholine failed to influence basal ANP-IR secretion (Fig. 2 *B*), but markedly inhibited norepinephrine-stimulated ANP-IR secretion (Fig. 7).

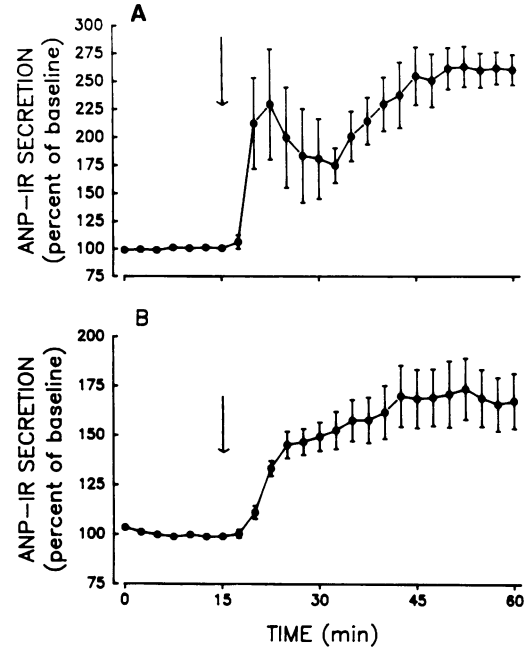

Figure 5. Effect of norepinephrine superfused with phentolamine or propranolol on ANP-IR secretion by rat left atria paced at 2 Hz. (*A*) Atria were continuously superfused with 10 μM phentolamine beginning 70 min before the addition of 10 μM norepinephrine (*arrow*) to the superfusate ($n = 3$ atria). Basal ANP-IR secretion was 177±38 pg/ml. (*B*) Atria were continuously superfused with 5 μM propranolol beginning 70 min before the addition of 10 μM norepinephrine (*arrow*) to the superfusate ($n = 6$ atria). Basal ANP-IR secretion was 245±39 pg/ml.

Discussion

Both α- and β-adrenergic agonists stimulate ANP secretion by isolated, paced rat left atria. However, the pattern of the secretory response by each is unique. The α_1-adrenergic agonist phenylephrine induces a monophasic rise in ANP secretion, whereas the β-adrenergic agonist isoproterenol produces a biphasic pattern of release. Also, the rapidity of the initial ANP secretory response differs quantitatively for α- and β-adrenergic stimuli. The initial secretory response to α-adrenergic stimulation is slower to develop relative to the β-adrenergic response. The differences in the atrial responses to phenylephrine and isoproterenol are further exemplified by the disparity in the onset and rate of rise of developed tension (11, 12, and Fig. 1). These observations suggest that α_1- and β-adrenergic agonists stimulate ANP secretion by different mechanisms. It is well recognized that the second messenger systems of α_1- and

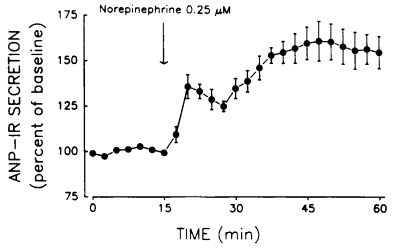

Figure 6. Effect of 0.25 μM norepinephrine superfusion on ANP-IR secretion by rat left atria paced at 2 Hz ($n = 5$ atria). Basal ANP-IR secretion was 320±15 pg/ml.

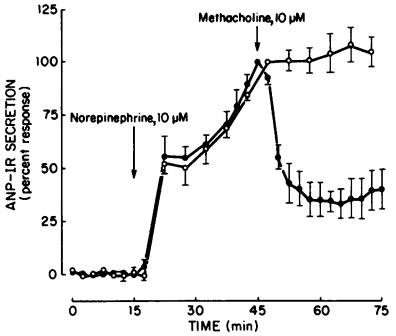

Figure 7. Effect of methacholine on norepinephrine-stimulated ANP-IR secretion. Rat left atria paced at 2 Hz were continuously superfused with 10 μM norepinephrine beginning at 15 min. 10 μM methacholine was added to the superfusate from 45 to 75 min (●, n = 5 atria). Control atria (○) were superfused with 10 μM norepinephrine alone (n = 2 atria) or 10 μM norepinephrine and 10 μM atropine with the addition of 10 μM methacholine at 45 min (n = 2 atria). The results are expressed as the net percent change in ANP-IR secretion from baseline with the response at 45 or 47.5 min defined as 100%.

β-adrenergic agonists are indeed distinct. α_1-Adrenergic agonists are known activators of the phosphoinositide pathway. β-Adrenergic effects are mediated by cAMP. Thus, the differences in the ANP secretory response to α_1- and β-adrenergic agonists may reflect the activation of unique second messenger systems.

Both α- and β-adrenergic agonists increase the cytosolic calcium concentration during systole which is, in part, responsible for the rise in developed tension (13, 14). The observations from this study suggest that ANP secretion is not solely due to the rise in cytosolic calcium. This statement is supported by two observations. First, the ANP secretory response to α- and β-adrenergic agonists are different. Second, ANP secretion is not proportional to developed tension. Methacholine alone dramatically lowered developed tension without changing ANP secretion. Methacholine also lowered the rise in developed tension by norepinephrine back to baseline, however ANP secretion did not fall to that level. Thus, the role of calcium as a second messenger of ANP secretion remains to be determined.

Norepinephrine, which possesses both α- and β-adrenergic activity, stimulates ANP secretion. The pattern of the ANP-IR secretory response to norepinephrine was similar to that of isoproterenol and dissimilar to the response pattern elicited by phenylephrine. This is consistent with the dominant β-adrenergic agonist effect of norepinephrine in cardiac tissues (11). However, norepinephrine also possesses the capability of stimulating ANP secretion by an α-adrenergic effect (Fig. 5 *B*). These observations suggest that norepinephrine-stimulated ANP-IR secretion reflects a predominance of β-adrenergic activity.

The effect of norepinephrine on ANP secretion in vitro has been previously reported to produce no change using statically incubated rat atria (15) or to have a stimulatory effect on secretion by the perfused rat heart (16). The secretory pattern of the norepinephrine-stimulated ANP response in the latter study was not thoroughly defined, since the time of perfusion with norepinephrine was not long enough to achieve a maximal response. In the present study, we were able to define the pattern of the secretory response to norepinephrine. Failure to observe an ANP secretory response to norepinephrine by Arjamaa and Vuolteenaho may be due to the inherent limitations in sensitivity using an incubation technique (15).

Epinephrine has been reported to stimulate ANP secretion in all (17, 18) but one study (15). The ANP secretory response to norepinephrine or epinephrine has been previously considered to be due to an α_1-adrenergic effect (16, 18). This conclusion was based on observations that α_1-adrenergic antagonists inhibit norepinephrine-stimulated ANP secretion, that phenylephrine stimulates ANP secretion, and that isoproterenol fails to stimulate ANP secretion. In the present study the pattern of the ANP secretory response to norepinephrine was similar to that of isoproterenol, not phenylephrine.

The differences between our study and previous studies are twofold. First, we found that isoproterenol stimulates ANP secretion. The reason that isoproterenol stimulated ANP secretion in this study is probably due to the fact that the frequency of atrial contraction was fixed. Previous investigators have primarily used the Langendorff heart preparation, where the frequency of contraction rose in response to isoproterenol. The rise in the frequency of contraction appears to alter the ANP secretory response to isoproterenol, as suggested by the observation that isoproterenol produces a delayed, smaller increase in ANP secretion when using spontaneously beating right atria in our system (Schiebinger, R. J., M. Z. Baker, and J. Linden, unpublished observations). The right atrial ANP secretory response to isoproterenol, which we observed, was similar to that reported for forskolin using the Langendorff heart preparation (19). Secondly, previous investigators have not been able to distinguish between an α- and β-adrenergic response as we have in this study due to a lack of carefully directed time course studies of the responses.

The muscarinic cholinergic agonist methacholine, when used alone, failed to influence ANP-IR secretion. However, when ANP-IR secretion was first enhanced by norepinephrine, methacholine inhibited ANP-IR secretion. Inhibition of norepinephrine-stimulated ANP-IR secretion by methacholine may be due, in part, to inhibition of adenylate cyclase activation by β-receptor agonist occupancy, a well-known property of muscarinic cholinergic agonists (20). The failure of the acetylcholine analogue methacholine in our study to increase ANP secretion is similar to one previous report (15). These results differ from two earlier reports where acetylcholine enhanced ANP secretion (17, 21). The biological activity of the methacholine in our study was demonstrated by a fall in developed tension and by inhibition of norepinephrine-stimulated ANP secretion. We cannot explain the discrepancy in our findings with those of other investigators except that methodological differences exist that may influence the results.

The collective results from our study suggest that the autonomic nervous system may influence ANP secretion in vivo. Activation of the sympathetic nervous system or a fall in parasympathetic tone may enhance ANP secretion. This may be one of the mechanisms whereby exercise increases plasma ANP in man (22–24). In contrast, a rise in activity of the parasympathetic nervous system may lower ANP secretion. The autonomic nervous system may also modulate the ANP secretory response to other stimuli, such as stretch, by increasing the secretory response due to an increase in sympathetic tone and lowering the response due to an increase in parasympathetic tone. However, Ledsome and colleagues concluded that sympathetic stimulation has no significant effect on ANP secretion in the anesthetized dog (25). It is not known what effects anesthesia and surgery have on ANP secretion in this animal model. Thus, the potential role of the autonomic ner-

vous system in modulating ANP secretion remains to be determined.

In summary, both α- and β-adrenergic agonists stimulate ANP secretion by rat atria paced at a fixed rate. The pattern of the ANP secretory response to α- and β-adrenergic agonists differ, suggesting that unique signaling pathways exist for each. Norepinephrine stimulates ANP secretion with a stimulatory response pattern similar to that of a pure β-adrenergic agonist. Methacholine does not influence basal ANP secretion but does inhibit norepinephrine-stimulated ANP secretion. Thus, the endogenous neurotransmitters of the autonomic nervous system norepinephrine and acetylcholine may influence ANP secretion in vivo by augmenting ANP secretion with an increase in sympathetic tone and lowering ANP secretion by elevating parasympathetic tone.

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