Biochemical Mechanism of Release of Atrial Natriuretic Polypeptide

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To define transmembrane and intracellular mechanisms of production and release of atrial natriuretic polypeptide (ANP) in the absence of mechanical atrial stretch, we studied the direct effects of physiological stimuli on isolated adult rat atrial myocytes maintained under tissue culture. Although stimulation of beta-adrenergic receptors on the surface of atrial myocytes by isoproterenol did not affect ANP release, adrenergic alpha-1 receptor stimulation by methoxamine enhanced ANP release with reciprocal intracellular ANP reduction. When muscarinic receptors were stimulated by acetylcholine, ANP release was accelerated and intracellular ANP reduced. The activation of the phosphatidylinositol system, which is a common pathway for muscarinic and alpha-1 adrenergic receptor stimulation, was thus considered to regulate ANP release, but not ANP production.

Electron micrograph of atrial myocytes from the mammalian heart shows numerous electron-dense cytoplasmic granules very similar to those in endocrine cells. These specific atrial granules were first described by Kisch in 1956, and their morphological features were characterized by Jamieson and Palade in 1964. However, it took more than two decades to clarify the physiological functions of these atrial granules. In 1981, deBold and associates demonstrated that crude extracts of rat atrial tissue produced a rapid and potent diuretic and natriuretic response when injected intravenously into anesthetized non-diuretic rats. With the development of biomedical research, the substance responsible for the natriuretic activity has been identified as a peptide (atrial natriuretic polypeptide, ANP), which has recently been purified and synthesized. Immune electron microscopy also showed that ANP was located in the atrial granules. If ANP is released into circulation in response to physiological stimuli, then it may play a significant role in fluid volume and blood pressure homeostasis. Current efforts are focused on the direct demonstration of the endocrine nature of ANP. Several experiments have revealed that ANP is released into circulation from the atria in response to an increase in atrial volume or atrial pressure. Although mechanical stimuli such as atrial muscle stretch induced by volume overload or atrial pressure increase can release ANP, the cellular mechanisms of regulation of ANP release are not yet clear. In this study, we demonstrated the biochemical mechanisms of ANP release from rat atria using cultured, isolated adult rat atrial myocytes.

MATERIAL AND METHODS

We previously established the methods to isolate atrial myocytes from adult rat hearts using enzyme perfusion techniques. Namely, 10–14 week old rats (Wistar strain, Charles River Japan, Inc.) were sacrificed by cervical dislocation. The heart was excised and immediately mounted onto the Langendorff perfusion apparatus. The entire process was performed under sterile conditions. The heart was perfused

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Key words:

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Experimental Protocol

Isolated atrial myocytes were resuspended in a culture medium equilibrated with 95% O₂ + 5% CO₂ at 37°C, pH 7.4.

- **Control medium**
- **Test medium**
- Centrifuged at 20g
- Culture medium
- Atrial myocytes
- Ultrasonic disruption
- Released ANP and intracellular ANP were measured by RIA

**Fig.1.** Experimental Protocol.

After one hour incubation, concentration of released ANP and intracellular ANP were measured by radioimmunoassay.

Sequentially with (i) a Ca²⁺ free buffer (Na⁺ 133 mM, K⁺ 3.8 mM and Mg²⁺ 1.2 mM) and (ii) a low Ca²⁺ (25 μM) buffer containing 0.125% trypsin and 1 mg/ml collagenase, both saturated with 95% O₂ + 5% CO₂. The right and left atria were carefully separated from the ventricles and were chopped into small cubes in the former solution, and atrial myocytes were dispersed with repetitive pipetting. The cell suspension was filtered through a stainless steel mesh with a pore size of 180 μm for removing larger debris, followed by a low speed centrifugation (20g) to separate small organella. Isolated atrial myocytes obtained in this manner were then resuspended and maintained in Eagle’s minimum essential culture medium at 37°C, pH 7.4. We studied the direct effects of four different physiological stimuli, namely, acetylcholine, isoproterenol, methoxamine and vasopressin, on these atrial myocytes. After 1 hour incubation without (control group) or with drugs (test groups) at either a low concentration of 10⁻⁶ M or a higher concentration of 10⁻⁴ M, the culture medium was centrifuged at a low speed (20g) for 1 min to separate atrial myocytes. ANP released into the culture medium and intracellular ANP were measured separately by radioimmunoassay using a tracer and an antiserum produced by Amersham (Fig. 1). The effects of three different osmolalities (280, 380 and 480 mOsm) of the culture medium on isolated adult atrial myocytes were also evaluated in the same fashion. Data were statistically analyzed with Student’s paired t-test, and P values less than 0.05 were considered significant.

**RESULTS**

1) Isolated adult rat atrial myocytes

Under a phase contrast microscope, striated structures were clearly visible in isolated adult rat myocytes. These myocytes were trypan blue negative, and represented viable cells. Electron microscopy showed numerous electron-dense granules around the nucleus and between myofibrils (Fig. 2, Fig. 3).

2) Effects of muscarinic receptor stimulation on ANP release into the culture medium (Fig. 4)

ANP release from atrial cells was significantly accelerated by acetylcholine at the concentration of 10⁻⁶ M (318 ± 101 pg/100 cells) (mean ± SD,

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pressin (10^{-6} M and 10^{-4} M), neither ANP release into the culture medium nor intracellular content was affected.

5) Effects of osmolalities on ANP release into the culture medium

Under three different culture medium osmolalities, neither ANP concentration in the culture medium nor that in the atrial myocytes was affected.

DISCUSSION

Using isolated perfused heart preparations Lang et al14 demonstrated that a rise in the right atrial pressure produced by increasing the perfusion rate resulted in an acceleration of ANP release. Veress et al13 reported that acute hypovolemia was associated with a release of ANP into the bloodstream and that removal of the atrial appendage reduced the amount of release. Atrial distension produced by mitral obstruction increased plasma ANP concentration.15 Although the effects of mechanical stimuli on ANP release have been extensively studied, biochemical mechanisms of either ANP production or ANP release have not been defined. We studied direct effects of acetylcholine, isoproterenol, metho- xamine, vasopressin and high osmolalities on isolated adult rat atrial myocytes. If the effects of these factors on ANP production or its release from the atria are evaluated in vivo, the data can be secondarily affected by the changes in hemodynamics caused by these factors. The methods established in our laboratory to isolate adult rat atrial myocytes are quite efficient in obtaining viable, trypan blue negative myocytes in good yield. A major merit to use isolated myocytes under culture conditions is that transmembrane and intracellular mechanisms can be directly evaluated in the absence of mechanical factors. Stimulation of muscarinic receptors by acetylcholine and of alpha-1 adrenergic receptors by methoxamine enhanced ANP release, but did not appear to affect intracellular ANP production. These results suggest that phosphatidylinositol response16 appears to be a common pathway for the acetylcholine and methoxamine actions in the stimulus-secretion coupling in adult atrial myocytes.

REFERENCES