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ENDOTHELIN IS A POTENT SECRETAGOGUE FOR ATRIAL NATRIURETIC PEPTIDE IN CULTURED RAT ATRIAL MYOCYTES

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Summary: Using cultured neonatal rat atrial cardiocytes, we have studied the effect of synthetic porcine endothelin (pET), a novel potent vasoconstrictor isolated from endothelial cells, on the release of immunoreactive (IR) rat atrial natriuretic peptide (rANP). pET stimulated IR-rANP secretion in a dose-dependent manner (10^{-10}-10^{-7}M) with an approximate half-maximally stimulatory dose of 2×10^{-10}M. The pET-induced IR-rANP secretion was attenuated by Ca^{2+}-channel blocker nicardipine, but no further stimulation was induced when combined with a Ca^{2+}-channel agonist BAY-K 8644. pET in combination with tetradecanoyl-phorbol-acetate resulted in a synergistic effect on IR-rANP secretion. These data suggest that ET may play as an endogenous secretagogue for rANP by modulating Ca^{2+} influx through the voltage-dependent Ca^{2+}-channels in atrial cardiocytes.

Atrial natriuretic peptide (ANP), a potent nariuretic and vasoactive polypeptide hormone, is synthesized and secreted by mammalian atrial myocytes (1,2). The release of ANP from cardiac atria is shown to be mainly stimulated by pressure-induced atrial distension and/or volume expansion (3,4). Using primary culture of neonatal rat atrial myocytes, we have recently demonstrated that synthesis of rat(r) ANP is regulat-

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ed by glucocorticoids and thyroid hormones (5), and that Ca^{2+} influx through the voltage-dependent Ca^{2+}-channel as well as activation of protein kinase C are closely involved in the secretory mechanism of rANP (6).

Recently, a novel vasoactive peptide termed endothelin (ET) has been isolated from the cultured media of porcine endothelial cells (7). Porcine(p) ET with a 21 amino-acid residues induces a potent vasoconstriction apparently dependent on extracellular Ca^{2+}, suggesting the involvement of Ca^{2+} influx via the voltage-dependent Ca^{2+} channel for its vascular action. Availability of synthetic pET prompted us to examine the effect of pET on immunoreactive (IR) rANP secretion from cultured rat atrial myocytes in vitro.

MATERIALS AND METHODS

Atrial myocytes from 2-4-day-old neonatal rats were dispersed by repeated digestion with 0.1% collagenase and pipetting, and cultured in Dulbecco's modified Eagles' medium (DMEM) supplemented with 20% fetal calf serum as described elsewhere (6). After 48 hrs, the monolayer myocytes ($\sim 10^5$ cells/dish) were rinsed and incubated in 1-ml DMEM containing 0.1% bovine serum albumin at 37°C for 60min. After incubation, medium was removed and assayed for IR-rANP by radioimmunoassay (RIA). Cell number was determined by a hemocytometer after trypsinization.

RIA was performed by the use of rabbit anti-α-rANP antiserum (final dilution, 1:30,000), ^{125}I-labeled-rANP as tracer, and α-rANP(1-28) as standard, essentially in the same manner as reported (6).

Synthetic pET and α-rANP were purchased from Peptide Institute (Osaka, Japan), 12-0-tetradecanoyl-phorbol-13-acetate (TPA) from Sigma Chemical (St. Louis, MO), BAY-K 8644 and nicardipine were kindly donated by Bayer Pharmaceutical Co. (Leverkusen, West Germany) and Yamanouchi Pharmaceutical Co. (Tokyo, Japan), respectively.

RESULTS

As shown in Fig. 1, pET dose-dependently (10^{-10}-10^{-7}M) stimulated IR-rANP secretion; a significant ($p < 0.05$) increase was observed with 10^{-10}M and a half-maximal stimulation was induced with 2×10^{-10}M. The pET-induced IR-rANP secretion

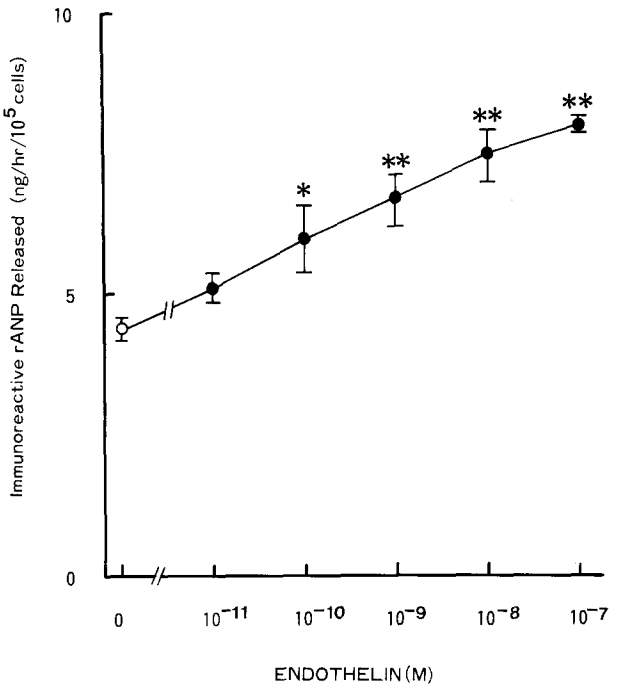
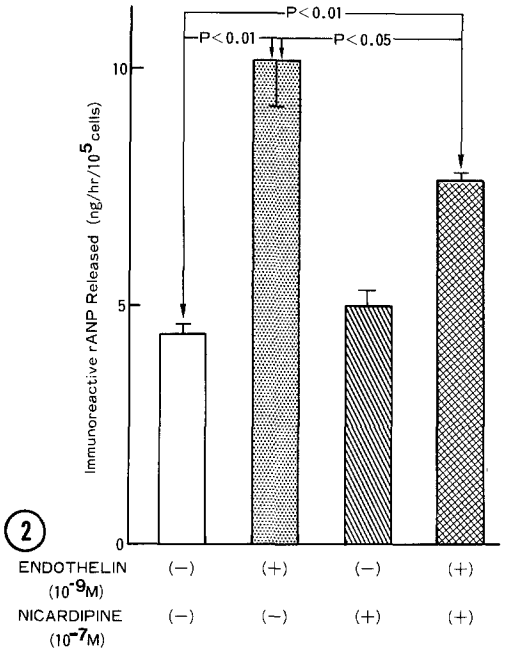


Fig. 1. Effect of porcine endothelin on rANP secretion from cultured rat atrial myocytes as a function of concentration. Rat atrial myocyte (10^5 cells) after 2-day-culture were incubated at 37°C for 60 min in the absence (o) or presence (●) of various doses of endothelin. Concentrations of immunoreactive rANP released into medium were determined. Each point is the mean of 3-5 dishes; bar indicates SE. Asterisks show statistically significant difference from control (* $P < 0.05$, ** $P < 0.01$).

was significantly ($p < 0.05$) attenuated in the presence of 10^{-7}M nicardipine, a voltage-dependent Ca^{2+}-channel blocker, whereas nicardipine alone did not affect the basal release of IR-rANP (Fig. 2). While BAY-K 8644 (10^{-6}M), a voltage-dependent Ca^{2+}-channel agonist, induced a significant increase in IR-rANP secretion, BAY-K combined with pET (10^{-9}M) resulted in no further increment (data not shown). TPA (10^{-8}M), a potent activator of protein kinase C, also augumented IR-rANP secretion and the combination of TPA and pET had greater stimulatory effect than did TPA or pET alone (Fig. 3), suggesting their synergistic effect on rANP secretion.



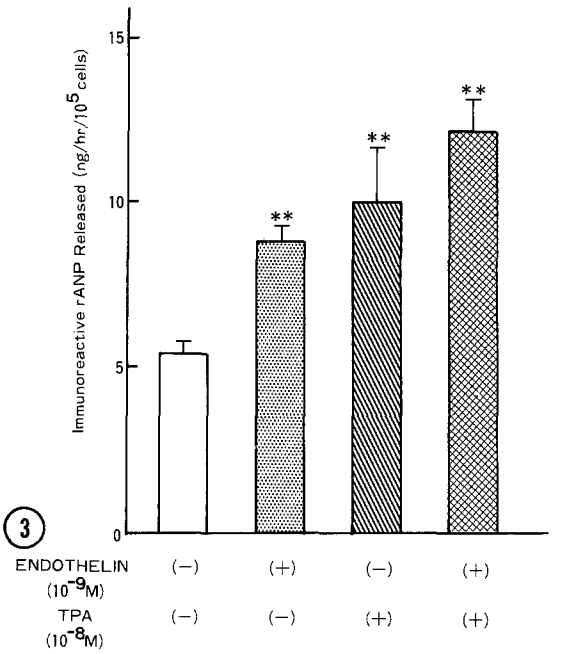


Fig. 2. Effect of nicardipine on endothelin-induced rANP secretion from cultured rat atrial myocytes.
Rat atrial myocytes in culture conditions same as in Fig. 1 were incubated in the absence (□) and presence of 10^{-9}M endothelin (▨), 10^{-7}M nicardipine (▧), or endothelin plus nicardipine (▩). Each column represents the mean of 3 dishes; bar shows SE. Statistical difference between each group was indicated by arrows.

Fig. 3. Effects of endothelin and TPA on rANP secretion from cultured rat atrial myocytes.
Rat atrial myocytes in culture conditions same as in Fig. 1 were incubated in the absence (□) and presence of 10^{-9}M endothelin (▨), 10^{-8}M TPA (▧), or endothelin plus TPA (▩). Data are plotted the same as in Fig. 2. (** $P < 0.01$)

DISCUSSION

pET, the most potent vasoconstrictor known to date, exerts its direct constrictive effect on the vascular smooth muscle, apparently distinct from actions by the well-recognized vasoconstrictors (7). In addition to its vasoconstrictive effect, pET has recently been shown to have positive inotropic and chronotropic action on isolated atria of the guinea pig (8).

The present study clearly demonstrates that pET is a potent secretagogue for rANP in cultured neonatal rat atrial

myocytes. The approximate half-maximal dose to stimulate rANP secretion by pET ($2x10^{-10}M$) is almost comparable to those of the ED_{50} ($4x10^{-10}M$) to induce vasoconstriction (7) and the apparent K_d ($2-4x10^{-10}M$) of ET receptors in cultured rat vascular smooth muscle cells (9).

The present study also shows that the pET-induced rANP secretion is attenuated by Ca^{2+}-channel antagonist, suggesting the possible involvement of Ca^{2+} influx through the voltage-dependent Ca^{2+}-channel in rANP secretion. Our data are also compatible with the requirement of extracellular Ca^{2+} for ET-induced vasoconstriction (7). Furthermore, BAY-K 8644 that directly acts on the voltage-dependent Ca^{2+}-channel (10) and stimulates rANP secretion (6), failed to induced further stimulatory effect in the presence of pET. These data suggest that pET and BAY-K act by the same mechanism, possibly on the membrane voltage-dependent Ca^{2+}-channels, to induce Ca^{2+} influx, thereby leading to the release of ANP.

We have recently demonstrated that TPA and Ca^{2+} ionophore ionomycin are both potent secretagogues for rANP in cultured rat atrial myocytes and show synergistic effect when combined together (6). The present result with synergism between TPA and pET on rANP secretion in conjunction with our previous observations (6) further lends a strong support to the notion that Ca^{2+} influx as well as protein kinase C play the important roles in the mechanism of ANP secretion, and suggest that ET may be involved in the regulation of ANP secretion, possibly as a endogenous Ca^{2+}-channel agonist or modulator in cardiocytes.

ANP is a newly-discovered cardiac hormone with a potent vasorelaxant effect (1,2), whereas ET is a novel endothelium-derived peptide with a potent vasoconstrictive effect (7).

Therefore, elucidation of the interaction between ANP and ET will open a new avenue to understanding the physiological and pathophysiological control of systemic blood pressure.

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