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Different Regulation of Atrial ANP Release through Neuropeptide Y_2 and Y_4 Receptors

Neuropeptide Y (NPY) receptors are present in cardiac membranes. However, its physiological roles in the heart are not clear. The aim of this study was to define the direct effects of pancreatic polypeptide (PP) on atrial dynamics and atrial natriuretic peptide (ANP) release in perfused beating atria. Pancreatic polypeptides, a NPY Y_4 receptor agonist, decreased atrial contractility but was not dose-dependent. The ANP release was stimulated by PP in a dose-dependent manner. GR 23118, a NPY Y_4 receptor agonist, also increased the ANP release and the potency was greater than PP. In contrast, peptide YY (3-36) (PYY), an NPY Y_2 receptor agonist, suppressed the release of ANP with positive inotropy. NPY, an agonist for $Y_{1,2,5}$ receptor, did not cause any significant changes. The pretreatment of NPY (18-36), an antagonist for NPY Y_3 receptor, markedly attenuated the stimulation of ANP release by PP but did not affect the suppression of ANP release by PYY. BIIE0246, an antagonist for NPY Y_2 receptor, attenuated the suppression of ANP release by PYY. The responsiveness of atrial contractility to PP or PYY was not affected by either of the antagonists. These results suggest that NPY Y_4 and Y_2 receptor differently regulate the release of atrial ANP.

Key Words : *Pancreatic Polypeptide; Peptide YY; Neuropeptide Y; Atrial Natriuretic Factor; Receptor; Contractility*

Feng Lian Piao*, Kuichang Yuan, Guang Yi Bai, Jeong Hee Han, Woo Hyun Park, and Suhn Hee Kim

Department of Physiology, Medical Sciences, Chonbuk National University Medical School, Jeonju, Korea; College of Nursing*, Yanbian University, Yanji, China

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Address for correspondence
Suhn Hee Kim, M.D.
Department of Physiology, Chonbuk National University Medical School, San 2-20 Keumam-dong, Deokjin-gu, Jeonju 561-180, Korea
Tel : +82.63-274-9892, Fax : +82.63-274-9788
E-mail : shkim@chonbuk.ac.kr

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INTRODUCTION

Pancreatic polypeptide (PP) and peptide YY (PYY) belong to the neuropeptide Y (NPY) family, which have well-conserved amino acid sequences (1) containing numerous tyrosines and tertiary structures (2, 3) with wide variation in anatomical distribution (4). The structural similarity between these peptides leads to the hypothesis that they are homologous, belonging to a family that has been termed the NPY family on the basis that NPY is evolutionarily the most ancient member. Five receptors for NPY family have so far been cloned, Y_1, Y_2, Y_4, Y_5, and y_6, and found to belong to the huge family of heptahelical G protein-coupled receptors (5). Y_4 receptor mRNA has been detected in the heart, gut, adrenal gland and artery (6-8). PP has a high affinity for Y_4 receptor whereas PYY and NPY have a low affinity for the Y_4 receptor (9, 10). PYY is as potent as NPY in activating Y_1, Y_2, and Y_5 receptors.

Among these peptides, PP expression is restricted to pancreatic endocrine cells, type F islet cells, in which PP is released into the circulation after ingestion of food to regulate pancreatic and gastric secretion, as well as gallbladder contraction (11). PYY is also expressed in both neurons of gastrointestinal tracts and endocrine cells, where it has an inhibitory effect on gastric motility and secretion (4). NPY is co-localized with noradrenaline in most sympathetic nerve fibers throughout the body (12). Several studies about cardiovascular functions of NPY family have been performed. Rat PP inhibits neurogenic vasoconstriction evoked by electrical stimulation through Y_4 receptor (4). In the mouse, NPY activates Y_2 receptor on the parasympathetic nerve terminal (13) and evokes potent vasoconstriction by activating Y_1 receptors. A recent study (14) showing slow heart rate and low mean arterial pressure as a result of reduced sympathetic activity in Y_4 receptor-knockout mice suggests that Y_4 receptor deletion disrupts autonomic balance within the cardiovascular system. Only a few reports about the effects of PP on cardiovascular function are available (14, 15). Therefore, the aim of the present study was to investigate the direct effects of PP on atrial dynamics and atrial natriuretic peptide (ANP) release and to identify its receptor subtypes using isolated perfused rat atria.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats, weighing 300-350 g, were obtained

from the Orientbio Inc. (Seoungnam, Korea), were housed throughout the experiments in a laminar flow cabinet, and were maintained on standard laboratory chow *ad libitum*. All experimental animals used in this study were performed under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University. Standard guidelines for laboratory animal care were followed.

Experimental procedures

Isolated perfused beating atria were prepared using a previously described method (16). In brief, the left atrium was dissected from the heart after killing and fixed into a Tygon cannula. The cannulated atrium was transferred into an organ chamber, immediately perfused with oxygenated HEPES buffer solution at 36.5℃, and paced at 1.3 Hz (duration 0.3 msec, voltage 40 V). The composition of the HEPES buffer solution was as follows (HEPES 10 mM, NaCl 118 mM, KCl 4.7 mM, $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 25 mM, glucose 10 mM, and bovine serum albumin 0.1%, pH 7.4). The pericardial buffer solution contained (^{3}H) inulin to measure the translocation of extracellular fluid (ECF). Intra-atrial pressure was recorded on a Physiograph (MK-IV, Narco Bio-systems INC., Houston, TX, U.S.A.) via a pressure transducer (Statham P23Db, Oxnard, CA, U.S.A.) and pulse pressure was calculated from the differences in systolic and diastolic intra-atrial pressures. After stabilization for 100 min, the perfusate was collected at 2-min intervals under 4℃.

Experiments were performed with four groups. Group 1 was atrium perfused with HEPES buffer (n=6) throughout the experiment. After stabilization, the perfusate was collected at 2-min intervals for 70 min. Group 2 was atrium perfused with human PP (10^{-8}M, n=6; 10^{-7}M, n=8; 3×10^{-7}M, n=7). PP, a NPY Y_4 receptor agonist, was introduced into the atrial lumen after a 10-min control period, and perfusate was collected for 60 min. Group 3 was atrium perfused with GR 23118 (NPY Y_1 receptor antagonist and Y_4 receptor agonist, 10^{-7}M, n=6), PYY (3-36) (NPY Y_2 receptor agonist, 10^{-7}M, n=7) or rat NPY (NPY $Y_{1,2,5}$ receptor agonist, 10^{-7}M, n=8). GR 23118, PYY or NPY was introduced into the atrial lumen after a 10-min control period, and perfusate was collected for 60 min.

Group 4 was atrium pretreated with an antagonist for Y_3 or Y_2 receptor. NPY (18-36) (3×10^{-7}M, n=7), an antagonist for NPY Y_3 receptor, or BIIE0246 (3×10^{-7}M, n=7), an antagonist for NPY Y_2 receptor was administered as a pretreatment at 20 min after the start of the perfusion. PP (10^{-7} M, n=7) or PYY (3-36) (10^{-7}M, n=7) was simultaneously infused with an inhibitor after a 10-min control period.

Measurement of ANP concentration

The concentrations of immunoreactive ANP in the perfusate were measured with a specific radioimmunoassay (RIA), as described previously (17). RIA was performed in Tris-acetate buffer (0.1 M, pH 7.4) containing neomycin (0.2%), ethylenediamine tetra-acetic acid (1 mM), soybean trypsin inhibitor (50 benzoyl arginine ethyl ester units/mL), aprotinin (200 kallikrein inhibiting unit/mL), phenylmethylsulfonyl fluoride (0.4 mg%), sodium azide (0.02%) and bovine serum albumin (1%). Standard and samples were incubated with anti-ANP antibody and (^{125}I) ANP for 24 hr at 4℃. Bound forms were separated from the free form using a charcoal suspension or second antibody. RIA for ANP was done on the day of experiments and all samples in an experiment were analyzed in a single assay.

We previously reported a two-step sequential mechanism of ANP secretion from the atrium (18, 19): first, atrial release of ANP into the interstitial space occurs by means of atrial stretching, and second, the released ANP is translocated into the atrial lumen, concomitantly with ECF translocation due to contraction. Therefore, the interstitial ANP concentration was calculated using the ANP secretion divided by extracellular fluid (ECF) translocation and 3060 (molecular mass for ANP-[1-28]) and was expressed in μM (18, 19).

Measurement of ECF translocation

The radioactivity of (^{3}H) inulin in the atrial perfusate was measured with a liquid scintillation counter (Tris-Carb 23-TR; A Packard Bioscience Company, Downers Grove, IL, U.S.A.). The amount of ECF translocated through the atrial wall was calculated from the total radioactivity in the perfusate divid- ed by the radioactivity in the pericardial reservoir and atrial wet weight and was expressed in μL/min/g.

Statistical analysis

The results are presented as the means±S.E.M. The statistical significance of the differences was assessed using analysis of variance followed by Duncan multiple range test. The critical level of significance was set at $p<0.05$.

RESULTS

Effects of PP on atrial contractility and ANP release

After stabilization for 100 min, the perfusate was collected five times every 2 min to serve as a control period and then PP was infused at a concentration of 10^{-8}, 10^{-7}, or 3×10^{-7}M. Fig. 1A shows the effects of PP on contractility, ECF translocation, ANP secretion, and ANP concentration with time. PP decreased atrial contractility (Fig. 1Aa). The secretion of ANP gradually increased in a dose-dependent manner (Fig. 1Ac) throughout the experiment without a change in ECF translocation (Fig. 1Ab). We have previously reported that the ANP released from atrial myocytes into the inter-

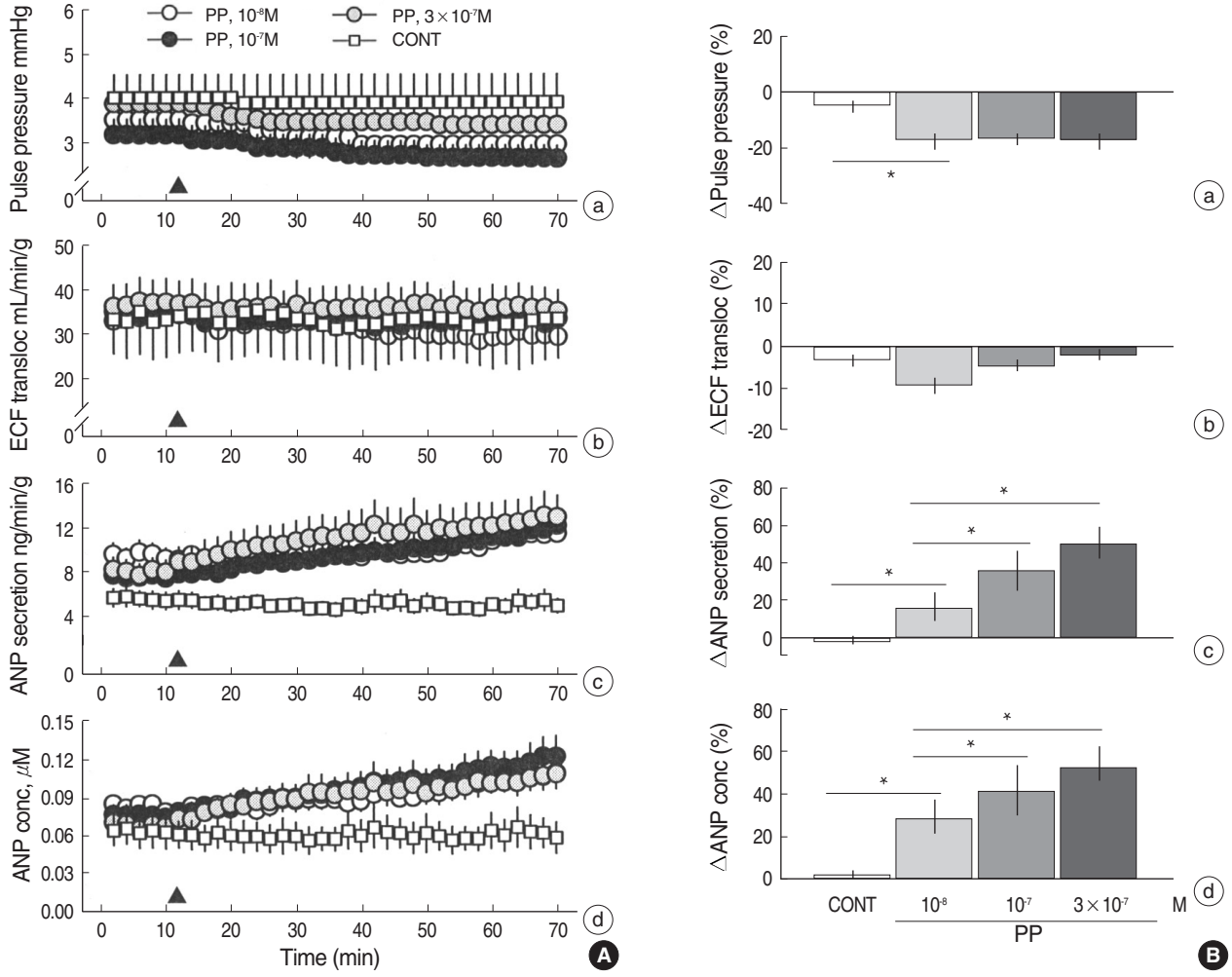

Fig. 1. (A) Effects of pancreatic polypeptide (10^{-8}M, n=6; 10^{-7}M, n=8; 3×10^{-7}M, n=7) on pulse pressure, ECF translocation, ANP secretion, and ANP concentration in isolated perfused beating rat atria. Pancreatic polypeptide decreased atrial contractility without change in ECF translocation. The ANP secretion and concentration gradually increased in terms of time. (B) Relative percent changes in pulse pressure, ECF translocation, ANP secretion, and ANP concentration by pancreatic polypeptide. Values were expressed as percent changes of the five peak experimental values for exposure to pancreatic polypeptide, as compared to the mean of the five control values. Pancreatic polypeptide increased the ANP secretion in a dose-dependent manner. Arrow indicates the start time of peptide infusion. PP, pancreatic polypeptide; CONT, control group; ECF transloc, ECF translocation; ANP conc, ANP concentration. *p<0.05; †p<0.01 vs. corresponding dose.

stitial space is translocated into the atrial lumen, concomitantly with ECF translocation (18, 19). The translocation of ECF is dependent on atrial contractility in this model. Therefore, the ANP secretion in terms of ECF translocation, which means the interstitial ANP concentration (Fig. 1Ad), was significantly increased by PP.

Fig. 1B shows the relative percent changes in pulse pressure, ECF translocation, ANP secretion and concentration obtained from the mean of five control values and the five peak experimental values (50-60 min after drug infusion) for exposure to the different doses of PP. PP decreased atrial contractility without dose-dependency (Fig. 1Ba) but ECF translocation did not change (Fig. 1Bb). The ANP secretion was increased by 16.52±7.29, 35.35±10.13, and 48.22±10.42% by 10^{-8}, 10^{-7}, or 3×10^{-7}M PP, respectively, which was dose-dependent (Fig. 1Bc).

Effects of GR 23118, PYY (3-36) and NPY on atrial contractility and ANP release

To compare the intra-atrial effects of GR 23118 (Y_1 antagonist and Y_4 agonist), PYY (3-36) (Y_2 agonist) and NPY ($Y_{1,2,5}$ agonist) with PP (Y_4 agonist), GR 23118, PYY (3-36) or NPY, at a concentration of 10^{-7}M, was perfused into atrial lumen. GR 23118 caused a gradual increase in ANP release and decreases in atrial contractility and ECF translocation (Fig. 2A). In contrast, PYY (3-36) abruptly decreased the ANP secretion and concentration, which maintained throughout the experiment. Atrial contractility and ECF translocation significantly increased (Fig. 2Aa, Ab). NPY

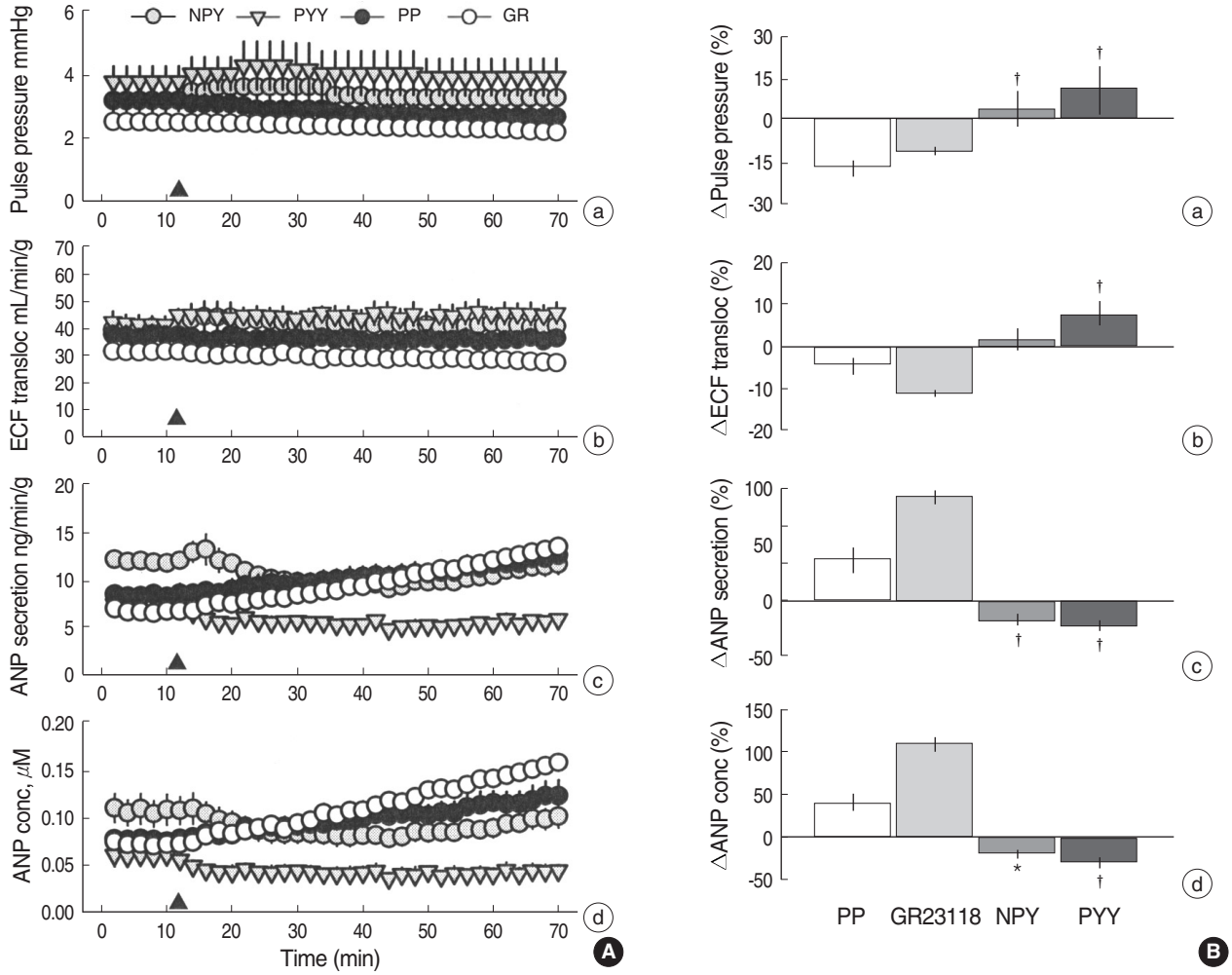

Fig. 2. (A) Effects of pancreatic polypeptide (PP, 10^{-7}M, n=8), GR 23118 (10^{-7}M, n=6), neuropeptide Y (NPY, 10^{-7}M, n=8), and peptide YY (3-36) (PYY, 10^{-7}M, n=7) on pulse pressure, ECF translocation, ANP secretion, and ANP concentration in isolated perfused beating rat atria. GR 23118 decreased atrial contractility and ECF translocation, and increased the ANP secretion. In contrast, peptide YY (3-36) increased atrial contractility and ECF translocation. The ANP secretion and concentration gradually decreased in terms of time. Neuropeptide Y also decreased the ANP secretion without significant changes in atrial contractility and ECF translocation. (B) Comparison of relative percent changes in several parameters between GR 23118, neuropeptide, peptide YY (3-36) and pancreatic polypeptide. Values were expressed as percent changes of the three peak experimental values for exposure to neuropeptide family, as compared to mean of the five control values. There was a significant difference in changes in ANP secretion by GR 23118, neuropeptide Y, and peptide YY (3-36), as compared to pancreatic polypeptide. Legends are the same as in Fig. 1. *p<0.05; †p<0.01 vs. pancreatic polypeptide-exposed group.

slightly decreased the ANP secretion until 30 min without significance (Fig. 2Ac). Atrial contractility and ECF translocation also did not change significantly.

Fig. 2B shows the comparison of relative percent changes in several parameters by GR 23118, PYY (3-36) and NPY with PP. GR23118-stimulated ANP secretion was greater than PP. The suppression of ANP secretion by NPY and PYY was significantly different from that by PP.

Modification of intra-atrial effects of PP and PYY (3-36) by receptor antagonists

To determine which Y receptor subtype(s) is(are) involved in PP-induced ANP secretion, NPY (18-36), Y_3 receptor antagonist, or BIIE0246, Y_2 receptor antagonist, was pretreated and PP or PYY (3-36) was simultaneously perfused. PP-induced negative inotropy was not affected by the pretreatment of NPY (18-36) or BIIE0246 (Fig. 3A). However, PP-stimulated ANP secretion was markedly attenuated by NPY (18-36) (Fig. 3C, D) but was not affected by BIIE0246. Both positive inotropy and suppression of ANP secretion by PYY (3-36) were not affected by NPY (18-36). PYY (3-36) -induced suppression of ANP secretion was attenuated by BIIE0246, an antagonist for Y_2 receptor.

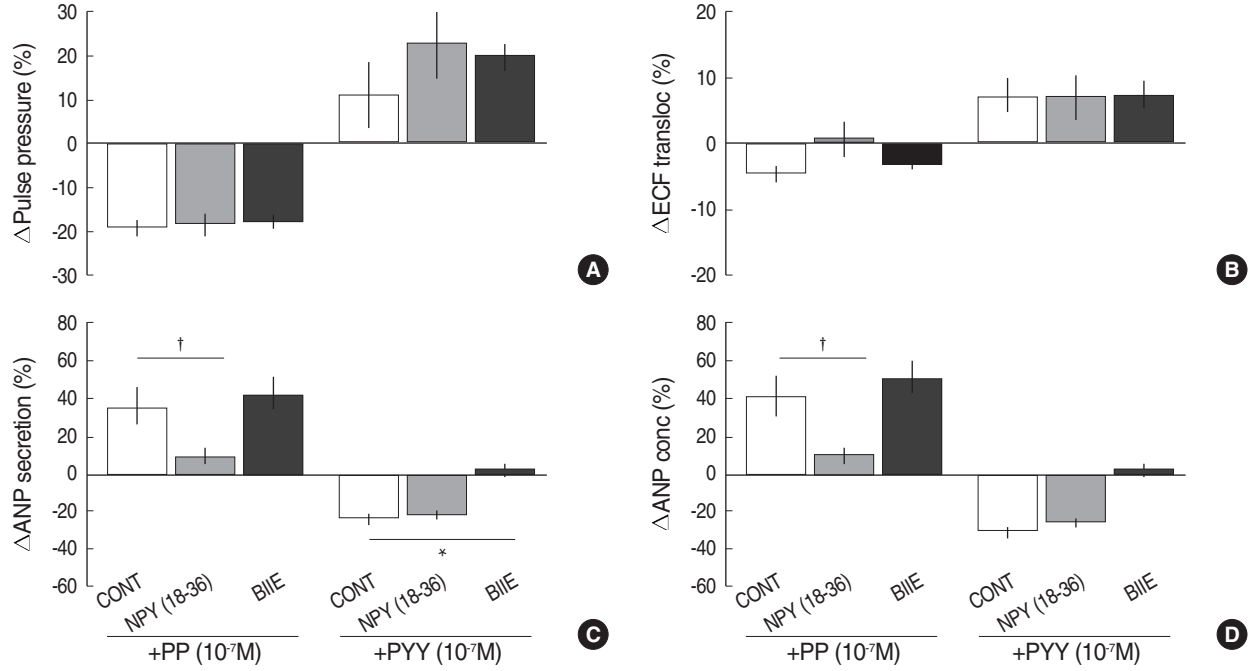

Fig. 3. Comparison of relative percent changes in pulse pressure (A), ECF translocation (B), ANP secretion (C), and ANP concentration (D) by pancreatic polypeptide (PP, 10^{-7}M, n=7) and peptide YY (3-36) (PYY, 10^{-7}M, n=7) in the presence and absence of neuropeptide (18-36) ($3x10^{-7}$M, n=7) and BIIE0246 (3×10^{-7}M, n=7). Pancreatic polypeptide-stimulated ANP secretion was attenuated by neuropeptide (18-36) and peptide YY (3-36)-suppressed ANP secretion was attenuated by BIIE0246. Legends are the same as in Fig. 1. *p<0.05; †p< 0.01 vs. the control group.

DISCUSSION

In the present study, we found that PP and GR 23118 (Y_4 receptor agonists) increased ANP secretion and PYY (3-36) (NPY Y_2 agonist) decreased ANP secretion. Therefore, we suggest that NPY receptors differently regulate the release of ANP.

PP, mainly produced in the gut, retains up to 50% residue identity to NPY or PYY, and a strong similarity in the C-terminal secretion. PP binds rat and mouse Y_4 receptor with very high affinity (7, 20), which is expressed in the periphery (21), including adrenal gland, gut, kidney (22) and heart. Although many studies on gastric actions of PP have been reported such as stimulation of gastric emptying, secretion and motility (23), a few report on cardiovascular function of PP are present (14, 15). In the present study, we investigated the intra-atrial direct effects of PP and PYY using isolated perfused rat atria. PP caused an increase in ANP secretion and negative inotropy. In contrast, PYY (3-36) caused a decrease in ANP secretion. It has been reported that PP has a high affinity for Y_4 receptor (24) and PYY (3-36) for Y_2 receptor (9). In the heart, Y_2 and Y_4 receptors have been shown to have an important role on cardiovascular functions. Rat PP inhibits neurogenic vasoconstriction evoked by electrical stimulation through Y_4 receptor (21) and transgenic Y_4-knockout mice show the significant impairment of cardiovascular function (14). We wanted to define which NPY receptor subtypes are involved in the regulation of ANP secretion and atrial contractility by PP and PYY (3-36). However, a specific Y_4 receptor antagonist is not available. Therefore, specific antagonists for Y_2 and Y_3 receptor subtypes were used. The PP-induced stimulation of ANP secretion was attenuated by Y_3 receptor antagonist but not by Y_2 receptor antagonist. PYY (3-36)-induced suppression of ANP secretion was attenuated by Y_2 receptor antagonist but not by Y_3 receptor antagonist. These results show a possible involvement of the Y_3 receptor for PP-induced ANP secretion. However, the Y_3 receptor to this day is unproven, and even scientists who originated the Y_3 concept have confirmed negative findings (especially with the CXCR4 chemokine receptor) related to the concept (25). It has also been reported that NPY (18-36) may act as an antagonist for Y_4 receptors (26). Additionally, GR23118, a specific Y_4 receptor agonist and Y_1 antagonist, stimulated the ANF secretion more than PP. Therefore, we suggest that NPY Y_2 and Y_4 receptors may differently regulate the release of atrial ANP.

In this study, PP caused negative inotropy and PYY (3-36) caused positive inotropy. This response was relatively small. Neither Y_2 nor Y_3 receptor antagonists modified the inotropic effects of PP and PYY (3-36). It is not clear that the hemodynamic changes by PP and PYY (3-36) are their own effects because of the small change and no observable dose-dependency. Because of the lack of inotropic effects by PP and PYY (3-36), the translocation of ECF was not sig-

nificantly changed. Therefore, we suggest that PP and PYY (3-36) lack direct atrial inotropic effects.

What is the physiological significance of PP-induced ANP release? It has been reported that following ingestion of food or hypoglycemia, PP is released from pancreatic F islet cells into circulation to regulate pancreatic and gastric secretion. We do not know the blood concentration of PP in hypoglycemic condition. However, it is possible that PP-induced ANP release may partly participate the ANP-dependent lipolysis (27) followed by an increased blood glucose level.

In conclusion, we suggest that NPY Y_2 and Y_4 receptors differently regulate the release of atrial ANP.

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