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, Y1, Y2, Y4, Y5, and y6, and found to belong to the huge family of heptahelical G protein-coupled receptors (5). Y4 receptor mRNA has been detected in the heart, gut, adrenal gland and artery (6-8). PP has a high affinity for Y4 receptor whereas PYY and NPY have a low affinity for the Y4 receptor (9, 10). PYY is as potent as NPY in activating Y1, Y2, and Y5 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 Y4 receptor (4). In the mouse, NPY activates Y2 receptor on the parasympathetic nerve terminal (13) and evokes potent vasoconstriction by activating Y1 receptors. A recent study (14) showing slow heart rate and low mean arterial pressure as a result of reduced sympathetic activity in Y4 receptor-knockout mice suggests that Y4 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 °C, 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, CaCl2 2.5 mM, MgSO4 1.2 mM, NaHCO3 25 mM, glucose 10 mM, and bovine serum albumin 0.1%, pH 7.4). The pericardial buffer solution contained (3H) inulin to measure the translocation of extracellular fluid (ECF). Intratra- vial 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 °C.

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 \times 10^{-7}M, n=7). PP, a NPY Y4 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 Y1 receptor antagonist and Y4 receptor agonist, 10^{-7}M, n=6), PYY (3-36) (10^{-7}M, n=7), or rat NPY (NPY Y1,2,5 receptor agonist, 10^{-7}M, n=7). PP decreased atrial contractility (Fig. 1Aa). The secretion of ANP gradually increased in a dose-dependent manner. PP decreased atrial contractility (Fig. 1Aac). Therefore, the interstitial ANP concentration (RIA) was measured in a single assay.

Measurement of ECF translocation

The radioactivity of (3H) 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 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 \times 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-
stitial space is translocated into the atrial lumen, concomi-
tantly with ECF translocation (18, 19). The translocation of
ECF is dependent on atrial contractility in this model. There-
fore, 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 pres-
sure, 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 × 10⁻⁷M 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 (Y₁ antag-
onist and Y₄ agonist), PYY (3-36) (Y₂ agonist) and NPY
(Y₁,₂,₅ agonist) with PP (Y₄ agonist), GR 23118, PYY (3-
36) or NPY, at a concentration of 10⁻⁷M, was perfused into
atrial lumen. GR 23118 caused a gradual increase in ANP
release and decreases in atrial contractility and ECF translo-
cation (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
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), Y1 receptor antagonist, or BIIE0246, Y2 receptor antagonist, was pre-treated and PP or PYY (3-36) was simultaneously perfused. PP-induced negative inotropy was not affected by the pre-treatment 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 Y2 receptor.
DISCUSSION

In the present study, we found that PP and GR 23118 (Y4 receptor agonists) increased ANP secretion and PYY (3-36) (NPY Y2 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 Y4 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 Y1 receptor (24) and PYY (3-36) for Y4 receptor (9). In the heart, Y1 and Y4 receptors have been shown to have an important role on cardiovascular functions. Rat PP inhibits neurogenic vasoconstriction evoked by electrical stimulation through Y1 receptor (21) and transgenic Y1-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 Y4 receptor antagonist is not available. Therefore, specific antagonists for Y2 and Y3 receptor subtypes were used. The PP-induced stimulation of ANP secretion was attenuated by Y3 receptor antagonist but not by Y2 receptor antagonist. PYY (3-36)-induced suppression of ANP secretion was attenuated by Y2 receptor antagonist but not by Y3 receptor antagonist. These results show a possible involvement of the Y3 receptor for PP-induced ANP secretion. However, the Y3 receptor to this day is unproven, and even scientists who originated the Y3 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 Y4 receptors (26). Additionally, GR23118, a specific Y4 receptor agonist and Y1 antagonist, stimulated the ANF secretion more than PP. Therefore, we suggest that NPY Y2 and Y4 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 Y1 nor Y4 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-
significantly 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 β cell islets 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 glycemic condition. However, it is possible that PP-induced ANP release may partly participate the ANP-dependent glycemic condition. Therefore, we suggest that PP and PYY differently regulate the release of atrial ANP.

In conclusion, we suggest that NPY Y1, Y2, and Y4 receptors differently regulate the release of atrial ANP.

REFERENCES

1. Larhammar D. Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. Regul Pept 1996; 62: 1-11.
2. Allen J, Novotny J, Martin J, Heinrich G. Molecular structure of mammalian neuropeptide Y: analysis by molecular cloning and computer-aided comparison with crystal structure of avian homologue. Proc Natl Acad Sci USA 1987; 84: 2532-6.
3. Mackerell AD Jr, Hemen A, Lacroix JS, Lundberg JM. Analysis of structure-function relationships of neuropeptide Y using molecular dynamics simulations and pharmacological activity and binding measurements. Regul Pept 1989; 25: 295-313.
4. Ekbland E, Sundler F. Distribution of pancreatic polypeptide and peptide YY. Peptides 2002; 23: 251-61.
5. Michel MC, Beck-Sickinger A, Cox H, Doos HS, Herzog H, Larhammar D, Quirion R, Schwartz T, Westfall T. XVI. Identification and distribution of mRNA encoding the Y1, Y2, Y4, and Y5 receptors for neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol Rev 1998; 50: 143-50.
6. Goumain M, Voisin T, Lorinet AM, Laburthe M. Localization and distribution of mRNA encoding the Y1, Y2, Y4, and Y5 receptors for peptides of the PP-fold family in the rat intestine and colon. Biochem Biophys Res Commun 1998; 247: 52-6.
7. Gregor P, Millham ML, Feng Y, DeCarr LB, McCaleb ML, Cornfield LJ. Cloning and characterization of a novel receptor to pancreatic polypeptide, a member of the neuropeptide Y receptor family. FEBS Lett 1996; 381: 58-62.
8. Whitcomb DC, Vigna SR, McVey DC, Taylor IL. Localization and characterization of pancreatic polypeptide receptors in rat adrenal glands. Am J Physiol 1992; 262: G532-6.
9. Bard JA, Walker MW, Branchek TA, Weinshank RL. Cloning and functional expression of a human Y4 subtype receptor for pancreatic polypeptide, neuropeptide Y, and peptide YY. J Biol Chem 1995; 270: 26762-5.
10. Walker MW, Smith KE, Bard J, Vaysses PJ, Gerald C, Daouti S, Weinshank RL, Branchek TA. A structure-activity analysis of the cloned rat and human Y4 receptors for pancreatic polypeptide. Peptides 1997; 18: 609-12.
11. Rogers RC, McGiggett DM, Herrmann GE. Vagal control of digestion: Modulation by central neural and peripheral endocrine fac-

tors. Neurosci Biobehav Rev 1996; 20: 57-66.
12. Lundberg JM, Terenius L, Hokfelt T, Goldstein M. High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci Lett 1983; 42: 167-72.
13. Smith-White MA, Herzog H, Potter EK. Cardiac function in neuropeptide YY receptor-knockout mice. Regul Pept 2002; 110: 47-54.
14. Smith-White MA, Herzog H, Potter EK. Role of neuropeptide Y Y(2) receptors in modulation of cardiac parasympathetic neurotransmission. Regul Pept 2002; 103: 105-11.
15. Kilbom MJ, Potter EK, McCloskey DJ. Neuromodulation of the cardiac vagus: comparison of neuropeptide Y and related peptides. Regul Pept 1985; 12: 155-61.
16. Han JH, Cao C, Kim SZ, Cho KW, Kim SH. Decreases in ANP secretion by lysophosphatidylcholine through protein kinase C. Hypertension 2003; 41: 1380-5.
17. Cho KW, Seul KH, Kim SH, Seul KM, Ryu H, Koh GY. Reduction volume dependence of immunoactive atrial natriuretic peptide secretion in isolated perfused rabbit atria. J Hypertens 1989; 7: 371-5.
18. Cho KW, Seul KH, Kim SH, Koh GY, Seul KM, Hwang YH. Sequential mechanism of atrial natriuretic peptide secretion in isolated perfused rabbit atria. Biochem Biophys Res Commun 1990; 172: 423-31.
19. Cho KW, Kim SH, Hwang YH, Seul KH. Extracellular fluid translocation in perfused rabbit atria: implication in control of atrial natriuretic peptide secretion. J Physiol 1993; 408: 591-607.
20. Lundell I, Statnick MA, Johnson D, Schober DA, Starback P, Gehlert DR, Larhammar D. The cloned rat pancreatic polypeptide receptor exhibits profound differences to the orthologous receptor. Proc Natl Acad Sci USA 1996; 93: 5111-5.
21. Barrios VE, Sun J, Douglass J, Toombs CF. Evidence of a specific pancreatic polypeptide receptor in rat arterial smooth muscle. Peptides 1999; 20: 1107-13.
22. Parker SL, Parker MS, Crowley WR. Characterization of Y1, Y2, and Y5 subtypes of the neuropeptide Y (NPY) receptor in rabbit kidney. Sensitivity of ligand binding to guanine nucleotides and phospholipase C inhibitors. Regul Pept 1998; 76: 127-43.
23. McGiggett DM, Rogers RC. Pancreatic polypeptide stimulates gastric motility through a vagal-dependent mechanism in rats. Neurosci Lett 1995; 188: 93-6.
24. Yan H, Yang J, Marasco J, Yamaguchi K, Brenner S, Collins F, Karbon W. Cloning and functional expression of cDNAs encoding human and rat pancreatic polypeptide receptors. Proc Natl Acad Sci USA 1996; 93: 4661-5.
25. Movafagh S, Hobson JP, Spiegel S, Kleinman HK, Zukowska Z. Neuropeptide Y induces migration, proliferation, and tube formation of endothelial cells bimodally via Y1, Y2, and Y5 receptors. FASEB J 2006; 20: 1924-6.
26. Parker MS, Lundell I, Parker SL. Pancreatic polypeptide receptors: affinity, sodium sensitivity and stability of agonist binding. Peptides 2002; 23: 291-303.
27. Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. J Pharmacol Exp Ther 2004; 308: 984-9.