Renal and cardiac neuropeptide Y and NPY receptors in a rat model of congestive heart failure

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Am J Physiol Renal Physiol 293: F1811–F1817, 2007. First published September 5, 2007; doi:10.1152/ajprenal.00191.2007.—Neuropeptide Y (NPY) is a 36-amino acid peptide that is widely distributed in the brain and peripheral tissues. It is involved in a variety of physiological functions, including cardiovascular, endocrine, and neuroendocrine activities. Neuropeptide Y (NPY), a potent vasoconstrictor, is also involved in the pathophysiology of congestive heart failure (CHF). The objective of this study was to elucidate the changes in the NPY system in heart failure using an experimental model of high-output CHF. We examined the changes in plasma NPY, as well as the concentration of NPY in several different sympathetically innervated tissues during the course of CHF. We also determined the expression of the Y1 and Y2 receptor genes, and whether this pattern of expression might account for differences in cardiac and renal NPY function in heart failure.
METHODS

Creation of an aortocaval fistula. Male Wistar rats (250–350 g) were anesthetized with 25 mg/kg ip injection of tribrothanol. An abdominal midline incision was made to visualize the abdominal aorta and vena cava. Aortocaval (A-V) fistulas were placed below the level of the renal arteries by the method established by Garcia and Diebold (7). Briefly, a 2-cm section of the vessels distal to the origin of the renal arteries was temporarily occluded (~30 s) with a pair of mini-clamps. At the midpoint between the two clamps, an 18-gauge needle was inserted through the wall of the aorta, advanced into the vena cava through the common wall between the vessels, and manipulated from side to side to widen the opening between the aorta and vena cava. The needle was then withdrawn, the aortic puncture was sealed with cyanoacrylate glue, clamps were removed, and abdominal incisions were closed. After the surgical procedure was completed (~10 min), the rats were given 0.2 ml of 40,000 U/ml penicillin and incisions were closed. After the surgical procedure was completed and blood loss was controlled from side to side to widen the opening between the aorta and vena cava. Aortocaval (A-V) fistulas were placed below the level of the renal arteries.

Drug infusion studies. A separate experimental group, rats underwent sham operations (the same procedure except for the needle puncture) and served as controls. All protocols were approved by the Animal Care and Use Committees of Georgetown University and in accordance with National Institutes of Health (NIH) guidelines.

Chronic metabolic balance studies. Rats housed in metabolic cages had their food intake, water intake, fecal weight measured daily. Sodium intake was calculated by multiplying the concentration of the electrolytes in the rat chow by the total amount of food eaten per day. Urinary sodium was determined by flame photometer (IL-943, Instrumentation Labs). Daily fluid balance was determined by subtracting the daily urine volume from the amount of water the rats drank per day. Animals were monitored for 6–8 days to determine the severity of CHF. Rats with A-V fistula, which also demonstrated physical signs of CHF (i.e., lethargy and dyspnea) and had sodium excretion below 0.50 meq/day, were considered to be in decompensated (severe) heart failure. Rats excreting more than 0.50 meq/day of sodium were considered to be in compensated (moderate) heart failure. At the end of the 6- to 8-day period, the three groups were sacrificed and blood samples were taken via aortic puncture for determination of plasma NPY concentration, and a variety of sympathetically innervated organs (heart, spleen, adrenal medulla, kidneys) were removed and weighed for analysis of tissue NPY.

RESULTS

Daily sodium excretion. Daily urinary sodium excretion is depicted in Fig. 1A. Sham-operated controls (n = 9) demonstrated a postsurgical reduction in sodium excretion for 1 day and were back excreting presurgical levels of sodium by postoperative day 2. In rats with compensated heart failure (n = 6), neither serum sodium excretion was significantly reduced for several days following A-V fistula surgery (average Na⁺ excretion days 1–4: COMP: 1.07 ± 0.23 vs. sham: 2.07 ± 0.23 meq/day, P = 0.04); however, by postsurgical day 5, COMP rats were excreting levels similar to the sham-operated controls (average Na⁺ excretion days 5–8: COMP: 2.52 ± 0.16 vs. sham: 2.35 ± 0.15 meq/day, P = 0.37). In contrast, sodium excretion was significantly reduced in rats with decompensated heart failure (n = 6) as daily sodium excretion never increased over 0.25 ± 0.22 meq/day throughout the experimental period (P < 0.001 vs. sham and COMP).

Rats with decompensated heart failure experienced many of the symptoms commonly associated with CHF, such as dys-
The average total wet heart weight in the COMP and DECOMP groups was significantly increased compared with either of the control groups (P < 0.01). Total heart weight-to-body weight ratio was used as a marker for relative cardiac hypertrophy, and only DECOMP rats demonstrated a significant increase in heart weight-to-body weight ratio compared with the sham control and COMP groups (P < 0.05; Fig. 1C).

A separate group of COMP animals was used for the acute infusion studies (DECOMP could not survive acute surgery). After the equilibration period, baseline MAP and RBF were measured in sham and A-V fistula (COMP only) groups. Both MAP (Fig. 1D) and RBF (Fig. 1E) were significantly decreased in the compensated A-V fistula rats compared with sham (P < 0.01).

**Plasma and tissue NPY.** The average immunoreactive NPY plasma concentrations in control and A-V fistula rats are shown in Fig. 2A. Interestingly, plasma NPY was 80% higher in all A-V fistula rats, regardless of severity of CHF, compared with control animals (P < 0.04). This is in sharp contrast to the observed changes in NPY in key tissues, which appear to mirror severity of heart failure.

The content of NPY in key tissues from healthy control rats is shown in Fig. 2B. The adrenal gland expressed the highest baseline concentration of NPY protein. The atria showed slightly higher baseline levels of NPY compared with the ventricles (P < 0.05). The NPY concentration found in the renal artery was similar to that in the ventricles. Tissue NPY concentrations in the kidney and spleen were similar, and less than that found in the ventricles.

The heart and the kidneys exhibited differential responses to heart failure. Figure 2C compares the atrial tissue levels of NPY between sham, COMP, and DECOMP rats. Both groups of rats in heart failure demonstrated significant reductions in atrial NPY concentration compared with sham rats, with the greatest decrease in tissue NPY found in the DECOMP rats (P < 0.05 vs. sham and COMP). In contrast, ventricular and renal NPY content was significantly reduced in DECOMP but not COMP rats, compared with sham (P < 0.05; Fig. 2, D and G). In spleen and adrenal glands from both COMP and DECOMP rats, there were similar reductions in NPY concentration, compared with tissues from sham rats (P < 0.03 vs. sham for both tissues; Fig. 2, E and F). There was no difference in NPY concentration in the renal arteries among the three groups (Fig. 2H).

**Changes in NPY-Y1 receptor gene expression.** Figure 3 shows the changes in the mRNA concentration of the NPY-Y1 receptor in the hearts and kidneys of A-V fistula rats. Given the relatively low yield from RNA isolation, whole heart tissue was processed for mRNA analysis, rather than separate processing for the atria and ventricles. NPY-Y1 receptor gene expression was reduced >50% in the hearts of rats with A-V fistula compared with the sham controls (P < 0.05; Fig. 3A). Similar reductions in Y1 receptor mRNA were seen in the kidney tissue of rats with A-V fistula (P < 0.05 vs. sham; Fig. 3B).

**Changes in NPY-Y2 receptor gene expression.** Figure 4 illustrates the changes in mRNA concentration of the NPY-Y2 receptor in the hearts and kidneys of A-V fistula rats. In contrast to what was seen with the NPY-Y1 receptor, the heart and kidney tissues taken from rats with A-V fistula demonstrated a dramatic increase in NPY-Y2 receptor mRNA expres-
sion, depending on severity of CHF. Compared with sham, NPY-Y2 receptor mRNA was increased 30- and 75-fold over control levels in COMP and DECOMP rats, respectively (**P < 0.05; Fig. 4A). Similar results were found in the kidney tissue, with significant 2.5- and 6-fold increases in COMP and DECOMP rats, respectively (**P < 0.05 vs. sham; Fig. 4B). C–H: NPY levels in tissues from control and A-V fistula animals. In heart and kidney tissues, NPY concentration decreased with severity of heart failure. NPY was also decreased with A-V fistula in the spleen and adrenal gland, while remaining unchanged in the renal artery. Sham (n = 5), COMP (n = 9), DECOMP (n = 6), **P < 0.05 vs. sham. #P < 0.05 vs. COMP.

**DISCUSSION**

Numerous studies have reported that the actions of NPY are altered in both animal models and in humans with CHF (15, 16, 28, 32). In healthy subjects and in animals, NPY via Y1 receptors acts as a vasoconstrictor, potentiating the effects of other like agents, such as NE and ANG II (4, 19, 21). In heart failure, NPY’s vasoconstrictive properties were found to be attenuated and the natriuretic/afterload reducing actions enhanced (1). In contrast, in healthy individuals and animals, Y2 receptors act primarily as inhibitory presynaptic receptors, inhibiting NE and NPY release (9); or as a stimulatory angiogenic postsynaptic receptor (36). Its role in CHF has never been addressed. Our present study demonstrates that in the high-output heart failure model, the NPY system and its Y1 and Y2 receptors in the heart and the kidneys are differentially regulated and appear to play a compensatory role.

The A-V fistula model of CHF exhibits cardiomegaly, depressed MAP and RBF, and produces both moderate (COMP, compensate and excrete sodium) and severe (DECOMP, retain sodium and expire) disease. The high-output CHF model used in this set of experiments revealed the changes in the NPY system, similar to those reported in human studies (15). Our analysis began with a characterization of this model of heart failure. As expected, two forms of disease were demonstrated, differentiated by the level of urinary Na⁺ excretion.
In general, rats subjected to an A-V fistula demonstrated an increase in total heart weight, a decrease in MAP and RBF (Fig. 1, C–E), an increase in plasma NPY concentrations, and a decrease in peptide levels in almost all major sympathetically innervated organs (Fig. 2). This, most likely, reflected a marked overall increase in sympathetic activity, known to accompany CHF (17). As hypothesized with heart failure, the discrepancy in NPY levels in plasma and tissue may be attributed to the potential exhaustion in NPY-producing capabilities in each organ, which may have released their NPY into the systemic circulation as a result of sympathetic hyperactivity (18). This resulted in a high plasma NPY levels compared with decreased NPY concentrations in all organs including adrenal glands.

Fig. 3. NPY Y1 receptor expression in heart failure. A: NPY Y1 receptor mRNA in the heart. Both COMP (n = 9) and DECOMP (n = 6) rats demonstrated a significant decrease in expression of Y1 mRNA compared with sham (n = 5). *P < 0.05 vs. sham. B: NPY Y1 receptor mRNA in the kidney. Both COMP (n = 9) and DECOMP (n = 6) rats demonstrated a significant decrease in expression of Y1 mRNA compared with sham (n = 5). *P < 0.05.

Fig. 4. NPY Y2 receptor expression in heart failure. A: NPY Y2 receptor mRNA in the heart. Both COMP (n = 9) and DECOMP (n = 6) rats demonstrate a significant increase in expression of Y2 mRNA, with DECOMP rats increasing expression beyond COMP rats. *P < 0.05 vs. sham (n = 5). #P < 0.05 vs. COMP. B: NPY Y2 receptor mRNA in kidneys. Both COMP (n = 9) and DECOMP (n = 6) rats demonstrate a significant increase in expression of Y2 mRNA, with DECOMP rats increasing expression beyond COMP rats. *P < 0.05 vs. sham (n = 5). #P < 0.05 vs. COMP.

Fig. 5. Effect of NPY, Y1R, and Y2R agonists on RBF in control and A-V fistula rats. A: effect of Y1 agonist on RBF. High-dose (400 ng/min) infusion of the Y1 agonist reduced RBF in the sham (n = 5) group but did not alter RBF in A-V fistula rats (COMP only, n = 15). *P < 0.05 vs. saline. B: effects of Y2 agonist on RBF. High-dose (400 ml/min) infusion of the Y2 agonist enhanced RBF in both sham (n = 5) and A-V fistula rats (COMP only, n = 15) compared with saline. *P < 0.05 vs. saline. #P < 0.0001 vs. saline. C: effects of NPY on RBF. High-dose (400 ml/min) infusion of NPY reduced RBF in both sham (n = 5) and A-V fistula rats (COMP only, n = 15). #P < 0.0001 vs. saline.
The present studies confirmed and extended previous reports by demonstrating a differential pattern of expression of NPY and its major receptors, Y1 and Y2, which may have important implications in the progression of CHF. Interestingly, only the DECOMP group displayed a decrease in ventricular NPY levels (Fig. 2D), consistent with what was reported for CHF patients (2, 15, 16): this was also observed in the kidney (Fig. 2G). In addition, our study demonstrated a negative association between cardiac NPY concentration and the severity of heart failure (Fig. 2, C and D). Unlike previously reported observations, however, our studies included an examination of renal NPY content in healthy and CHF animals.

With regard to the kidney, high renin levels have been associated with the development of severe CHF. Interestingly, only rats in severe heart failure demonstrated a reduction in kidney NPY concentration (Fig. 2G). Since renal NPY depletion, like that in the heart, may indicate excessive NPY release from hyperactive sympathetic nerves, these data suggest that renal NPY may contribute to the severity of heart failure in these rats. However, previous studies showed NPY to reduce the release of renin in high-renin states like CHF (22, 31). Furthermore, Winaver et al. (30) showed that rats in severe heart failure display markedly increased plasma renin-angiotensin levels, while rats in moderate heart failure do not. Thus we hypothesize that while increased release of NPY in the kidneys may be beneficial, rats that exhausted their renal stores of NPY are unable to inhibit renin release, allowing for increased ANG II to further impair cardiovascular function.

In addition to determining the NPY content in different tissues, this study also examined the mRNA expression of NPY-Y1 and NPY-Y2 receptors in the hearts and kidneys of rats with moderate and severe heart failure. mRNA of both the Y1 and Y2 receptor was altered in rats with CHF. Y1 mRNA was significantly reduced in both the hearts and kidneys of COMP and DECOMP rats, dependent on severity of CHF (Fig. 3). This was similar to what is known to occur with β1-adrenergic receptor mRNA in patients with CHF (3), suggesting that both may reflect receptor downregulation due to enhanced release of sympathetic neurotransmitters during CHF-induced sympathetic activation. Conversely, the expression of Y2 receptor mRNA was marked increased in the heart and kidneys of rats with CHF (Fig. 4). This suggests that while the effects of Y1 receptors are attenuated in both the heart and the kidney, at the same time, the effects of Y2 receptors are enhanced.

Subsequently, we investigated whether these changes in receptor mRNA expression were associated with alterations in kidney function.

In a separate group of CHF rats, we studied the renal blood responses to specific NPY-Y1 and -Y2 receptor agonists. Animals that underwent an A-V fistula, whose basal RBF was lower than in control rats, exhibited an attenuated response to the RBF-lowering effects of Y1 agonist, [Leu31, Pro34]-NPY (Fig. 5A), presumably due to a downregulation of Y1 receptors. In contrast, the Y2 agonist (NPY3–36) tended to increase RBF (Fig. 5B) in CHF as well as in sham, suggesting functional Y2 receptors.

The differential changes in Y1 and Y2 receptor expression may be an important counterregulatory response to limit the excessive vasoconstriction seen in CHF by 1) a reduction in Y1 receptor-mediated vasoconstriction, direct and indirect via other vasoconstrictors such as NE or ANG II, 2) enhancement of the angiogenic effects of NPY via postsynaptic endothelial Y2 receptors (36) in peripheral and coronary vasculatures, and 3) an anti-adrenergic effect of NPY via presynaptic Y2 receptors to reduce NE release and hence, limit its deleterious activity in CHF. Although the expression of mRNA and protein level can be used as preliminary indicators of NPY receptor’s functionality in CHF, further studies are needed to investigate NPY signaling pathways and functional consequences in CHF.

It is interesting to note that NPY itself decreased RBF in CHF kidney (Fig. 5C), even in the presence of increased Y2 receptor expression. A likely explanation for this is the involvement of another receptor type, the Y5 receptor. As reported by our group (14, 23), the Y5 receptor, which is induced by tissue ischemia and injury (12), has amplifying effects on Y1 receptor-mediated mitogenic effects of the peptide (23). Vascular hypertrophy has previously been shown to increase responsiveness of the vessels to vasoconstrictors via purely structural reasons (Folkov’s theory) (5). Therefore, when NPY, a nonspecific Y1, Y2, and Y5 agonist, was infused, it might have been still effective in reducing RBF in CHF kidney by utilizing its Y5 receptors, despite the significant decrease in Y1 receptor expression. Thus, an overall effect of NPY in the renal circulation in CHF may be a net effect of enhanced vasoconstriction, vascular hypertrophy, and increased blood flow via Y2 receptors, either due to vasodilation and increased angiogenesis.

An alternative or additional possibility is that the changes in NPY receptor populations in this model of CHF are also associated with alterations in NPY metabolizing enzymes, specifically dipeptidyl peptidase IV (DPPIV). It has been shown that NPY (3–36), the end product following cleavage of NPY by DPPIV, is an endogenous Y2/Y5 receptor-preferring ligand (8). If this enzyme is upregulated along with the Y2 receptors, as we previously showed in other ischemic states (13), then subjects with CHF would have an innate mechanism for controlling excessive NE and renin release. Overall, characterizing the role of DPPIV and other NPY receptors in CHF would be useful to determine NPY’s role in CHF.

The heart failure-induced differential regulation of the NPY system suggests that NPY is an important neurovascular modulator in the development of CHF-related pathology. Further studies are needed to determine the factors contributing to whether NPY acts to compensate or exacerbate the development of the disease; as it is capable of deteriorating the conditions with excessive vasoconstriction and cardiac hypertrophy via Y1 receptors and enhancing the cardiovascular compensatory mechanisms, such as angiogenesis via Y2 receptors.

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REFERENCES


