

B-Type Natriuretic Peptide Decreases Gastric Emptying and Absorption

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Natriuretic peptides have been shown to decrease contractility of isolated gastric smooth muscle cells. However there is a paucity of research showing whether this effect has functional significance in the whole animal. The objective of this study was to test whether intravenously administered B-type Natriuretic Peptide (BNP) has an effect on gastric emptying and/or absorption in a whole animal mouse model. C57BL/6-Wild-type (WT) and Natriuretic Peptide Receptor type A (NPR-A) knockout (KO) mice were used in these studies. Gastric contractility was examined in anesthetized mice before and after BNP vs. vehicle injection. Gastric emptying of gavage fed 70 Kilo Dalton (kDa) FITC-dextran and absorption of 4 kDa FITC-dextran were compared in BNP vs. vehicle treated conscious WT and KO mice. BNP decreased gastric contractility (measured in change in intragastric pressure) from 2.26 ± 0.29 to 1.44 ± 0.11 mmHg ($P < 0.05$), pressure returned to 2.08 ± 0.17 after 5 BNP half-lives ($P < 0.05$). There was no significant change in the vehicle or KO. BNP also decreased gastric emptying in WT mice compared to vehicle, $87.8 \pm 0.8\%$ vs. $97.3 \pm 1.04\%$ ($P < 0.05$) and this effect showed a dose-response relationship. In KO mice emptying was $95.8 \pm 0.5\%$ (BNP) vs. $91.7 \pm 0.7\%$ (Vehicle) ($P > 0.05$). The absorption in WT mice was 28.2 ± 7.8 (relative fluorescence units) for BNP vs. 91 ± 25.9 for vehicle ($P < 0.05$). For KO mice absorption was 64.3 ± 14.9 for BNP vs. 60.6 ± 17.4 for vehicle ($P > 0.05$). The results show that BNP decreases intragastric pressure, emptying and absorption by acting via the NPR-A receptor. We postulate that this effect is aimed at decreasing preload through decreased water and electrolyte absorption from the GI tract and may also be responsible for the symptoms

of impaired gastrointestinal function observed in heart failure patients. *Exp Biol Med* 233:475–482, 2008

Key words: BNP; gastric emptying; absorption; heart failure; endocrine heart

Introduction

Although primarily associated with cardiac myocytes, B-type natriuretic peptide (BNP) and the family of natriuretic peptides are expressed in and released from non-cardiac tissue. Natriuretic peptide receptors also are expressed in non-cardiac tissue. In the gastrointestinal (GI) tract, natriuretic peptides are expressed in specific regions of the stomach and intestine where there is evidence that they are involved in paracrine regulation of secretory functions (1–4). Recent studies have also shown that Atrial Natriuretic Peptide (ANP) produced by the enterochromaffin cells of the gastric mucosa is released into the capillaries suggesting a possible endocrine function (5, 6). Since the major receptor for both ANP and BNP is the same guanylyl cyclase A (also known as NPR-A), high circulating levels of BNP released from the heart in states such as heart failure could have an effect on motor and secretion functions in the GI tract. Such an effect by circulating natriuretic peptides, particularly BNP, could have a modulating function on the gut and could be responsible for some of the symptoms observed in people with high plasma BNP levels, as in the case of heart failure patients. In addition, natriuretic peptides may modulate volume status and preload (i.e., the pressure stretching the cardiac myocytes or the ventricle) by way of influencing water and electrolyte absorption from the GI tract.

Prior studies have shown that natriuretic peptides relax isolated gastric smooth muscle cells in different species including humans (7–9). However there is a paucity of *in vivo* studies that have tested whether the effect on isolated smooth muscle cells has a functional significance in the whole animal. In view of the prevalence of GI symptoms in

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humans with heart failure (10), it is of interest to know whether pharmacologically administered or endogenously produced BNP has an effect on gastrointestinal function.

The objective of this study was to examine the effect of intravenously administered BNP on intragastric pressure, gastric emptying and absorption in a whole animal mouse model. We hypothesized that BNP would decrease gastric emptying by acting through the NPR-A receptor, which increases intracellular cyclic 3', 5'-guanosine monophosphate (cGMP) and relaxes smooth muscle cells. NPR-A knockout mice were used to rule out a non-specific response. The other members of the natriuretic peptide family of hormones, ANP and C-type natriuretic peptide (CNP) were also tested to establish whether the GI effect is common to the group or specific to one or more peptide; c-ANP₄₋₂₃, a specific agonist of natriuretic peptide receptor C (NPR-C), was used to determine if the GI effects of BNP are mediated through this alternative receptor pathway.

Methods

Experimental Animals. NPR-A Knockout (KO) mice were obtained from our resident colony that was founded with pathogen-free breeding pairs and were genetically monitored by PCR of tail-snip DNA. The generation of NPR-A knockout mice has previously been described in detail (11). Wild-type (C57BL/6) mice were purchased from commercial sources. The wild-type mice were all males with ages ranging from 8 to 12 weeks and weight ranging from 18–26 grams at the time of the experiment. The knockout mice ranged from 10 to 32 weeks in age and 24–38 grams in weight at the time of the experiment, with equal number of male and female KO mice in the experiment and vehicle group. The parental strain of the knockout mice was C57BL/6.

Surgical Preparation

Measurement of Intragastric Pressure. The mice were anesthetized with sodium pentobarbital (0.9 mg/10 gm body weight) given intraperitoneally (ip) and supplemental doses of 0.5 mg given ip as needed. The mice were placed on a temperature controlled surgical table and a tracheotomy was performed using a 20 ga. Luer-stub adapter. The right jugular vein was catheterized with polyethylene (PE) 10 tubing for injections and infusions and the right carotid was catheterized with a 5 cm piece of PE tubing (o.d. 0.012", i.d. 0.006": Braintree Scientific, Inc.) attached to a 12–18" segment of PE 50 tubing for arterial blood pressure measurements. A 10 mm left subcostal skin incision was made to access the stomach. A 3 mm vertical incision of the stomach fundus was made with cautery carefully choosing a site that has minimal or no visible blood vessels. A 2–3 mm latex balloon fitted with PE tubing and primed with saline was inserted into the stomach. The balloon was then minimally distended by adding 20–25 µl of saline and attached to a standard pressure transducer (Gould/Statham

DB25). The intragastric catheter was held in place by the minimal distension of the balloon and suturing the stomach incision was not necessary. The skin incision was closed with 1–2 interrupted surgical sutures. Following these surgical preparations, the mice received a 100 µl bolus of 0.9% saline intravenously (iv) via the jugular catheter and then allowed a one hour equilibration period while being infused with 0.9% saline iv at 5 µl/minute. The saline bolus and infusion were administered to compensate for blood loss associated with the surgical procedure and maintenance fluid requirement. Arterial blood pressure, heart rate and intragastric pressure were monitored continuously via the carotid and intragastric catheters and recorded on a data acquisition system (DATAQ Instruments, Akron, OH). The change in intragastric pressure was measured as the difference between the peak and baseline pressure. The measurements were taken for three 30-minute periods. The basal gastric pressure was measured from 30 to 0 minutes before injection. Post injection period was from 10 to 40 minutes after injection to correspond with the peak plasma level of the peptides and the recovery period was from 90 to 120 minutes after injection corresponding to the period later than 5 peptide half-lives (12). The changes in intragastric pressure during each of the three periods were averaged for each mouse and the differences in intragastric pressure between the experimental and vehicle groups during the three periods were compared using a one way ANOVA with Fisher's least squared difference test (LSD) used as a post hoc test. A *P* value of < 0.05 was considered the criteria for statistical significance.

Positive and Negative Controls. Ghrelin, a hormone with known prokinetic gastrointestinal effect in rodents, was used as a positive control to ascertain that the waves of contraction we observed and recorded were indeed changes in gastric motility (13). Ghrelin (Rat, Phoenix Pharmaceuticals, C# 031–31, Lot # 423341) was administered at a dose of 50 µg/kg body weight in 100 µl of vehicle iv, a dose previously established to increase motility in rodents. The vehicle was used as a negative control.

Blood Pressure. Mean arterial pressure was continuously monitored using an intra-carotid catheter and recorded. Average blood pressure was measured for the same three periods as for the gastric pressure measurement. Pre vs. post peptide injection blood pressures were compared to ascertain that our findings were not confounded by differences in blood pressure.

Measurement of Gastric Emptying. Conscious WT and KO mice (*n* = 4 in each group) were given BNP at dose of 10 ng/g through the tail vein dissolved in 100 µl of vehicle (modified Krebs solution) or the vehicle alone and immediately gavaged with 0.1 ml of 0.5 mmol 70 kDa FITC-dextran. Thirty minutes later, the animals were sacrificed and the stomach was separated and the intestine divided into eight equal segments, each flushed with 3 ml of PBS buffer and centrifuged for 10 minutes. Fluorescence of the supernatant fluid was measured and the percent gastric

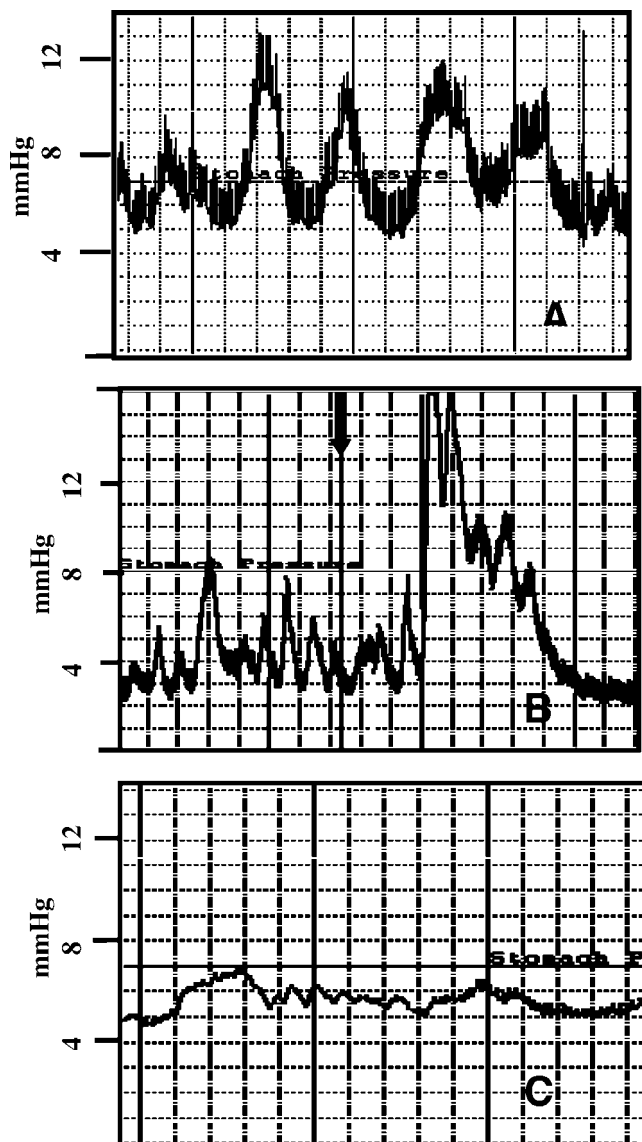


Figure 1. A typical basal gastric contraction wave pattern in wild-type mice is shown in Figure 1A. Contraction frequency averaged 3–7/minute with amplitudes ranging 0.5–10 mmHg. Intravenous administration of the prokinetic agent ghrelin resulted in a marked increase in the intragastric balloon pressure (B). Administration of BNP (10 ng/g) intravenously resulted in a marked attenuation of intragastric pressure as shown in Figure 1C.

emptying rate was compared in BNP treated vs. control for both WT and KO mice. This method of evaluating gastric emptying has been previously established (14).

Measurement of Absorption (Gastrointestinal Permeability). Conscious WT and KO mice ($n = 4$ in each group) were given 10 ng/g of BNP through the tail vein dissolved in 100 μ l of vehicle (modified Krebs solution) or the vehicle alone. The mice were then gavaged with 0.01 ml/g of a solution containing 22 mg/ml of 4 kDa FITC-dextran. Blood was collected via cardiac puncture under pentobarbital anesthesia. The plasma fluorescence measured

1 hour after gavage in WT vs. KO mice was compared for BNP treated vs. control. The group differences were analyzed using a Student's t test at $P < 0.05$. This method of evaluating gastric absorption has been previously established (14).

We also compared the fluorescence of a 50 μ l plasma sample taken 1 hour after iv administration of 100 μ l of 0.5 mmol 4kDa FITC-dextran in BNP treated vs. vehicle WT mice. The purpose of this test was to rule out the possibility that the changes in plasma fluorescence were produced by other actions of BNP such as increased excretion, redistribution or metabolism of the dextran.

The concentration of fluorescein was determined using a fluorimeter (FLUOstar Galaxy, BMG Labtechnologies) with an excitation wavelength at 485 nm and an emission wavelength of 520 nm using serially diluted samples of the marker as standard.

Plasma BNP levels were measured by radioimmunoassay (Peninsula Laboratories, 5–2104 RIAS 9085).

The peptides infused in the study included: ANP, (Rat ANP, Sigma, P # A8208), BNP (Rat, BNP-32, Phoenix Pharmaceuticals, 011–14, Lot # 421752), CNP (Rat 32–53, Bachem, P# H –1296, Lot # B00656) and c-ANP_{4–23} (Rat, c-ANP_{4–23}, Phoenix Pharmaceuticals, 005–26, Lot # 422388).

The peptides were administered iv at 10 ng/g body weight. This dose was chosen to raise and sustain the plasma levels above 500 pg/ml; a level that is consistent with a greater than 90% likelihood of heart failure (for BNP) in humans (15). For subsequent dose response experiments we administered BNP at 1, 5, 10 and 100 ng/g body weight iv.

This protocol was approved by the University of South Florida Institutional Animal Care and Use Committee.

Results

Intragastric Pressure. A typical intragastric pressure wave pattern is shown in Figure 1A; gastric contraction frequency averaged 3–7 times per minute ranging from 0.5 to 10 mmHg in amplitude. We used the prokinetic agent ghrelin to ascertain that our recording from the intragastric balloon corresponded to changes in gastric motility. Administration of ghrelin resulted in a marked and significant increase in intragastric balloon pressure, validating our measurement. Ghrelin was administered in three experiments and a typical response is shown in Figure 1B.

Figure 1C shows a typical gastric pressure wave pattern after administration of BNP. Gastric pressure was attenuated without significant change in frequency of contraction. A similar effect was observed for ANP and CNP (data not shown). This effect was not seen in NPR-A KO mice or with the specific NPR-C agonist, c-ANP_{4–23}.

We quantified the change in intragastric pressure by averaging the difference between the peak and baseline gastric pressures for three 30-minute periods; before,

immediately after and later than 5 peptide half-lives after peptide injection. Because each experimental animal had different baselines, comparing the difference between baseline and peak (i.e., the change in amplitude of the gastric contraction wave) was found to be more precise and reliably comparable among different experimental animals.

Figure 2 shows the pooled data on comparison of the average gastric pressure before peptide injection (Basal), immediately following peptide injection and later than 5 BNP half lives (Recovery) vs. vehicle. As shown, BNP significantly decreased intragastric pressure from a basal value of 2.26 ± 0.29 mmHg to 1.44 ± 0.11 mmHg and gastric pressure returned to 2.08 ± 0.17 mmHg when measured later than 5 BNP half-lives ($n = 5$, $P < 0.05$, ANOVA, Fisher's LSD test). Similar and statistically significant reduction of gastric pressure was obtained for ANP and CNP (Fig. 2B, C). Average gastric contractions per minute were 4.4 ± 0.7 , 3.1 ± 0.4 and 4.3 ± 0.5 for periods of BNP injection compared to 5.3 ± 1.2 , 4.2 ± 0.6 and 4.3 ± 0.5 for the vehicle group (all $P > 0.05$, ANOVA). There was no difference in gastric pressure in BNP treated vs. vehicle treated NPR-A KO mice.

Mean arterial blood pressure was continuously monitored during all experiments using an intra-carotid catheter. There was a slight reduction of average blood pressure from 62.9 ± 4.7 mmHg prior to BNP injection to 59 ± 3.2 mmHg ($n = 5$, $P > 0.05$, ANOVA, Fisher's LSD test). Blood pressure returned to 61.4 ± 5.08 mmHg when measured later than 5 peptide half-lives.

Gastric Emptying. Gastric emptying was measured as the percentage of 70 kDa FITC-Dextran that had emptied out of the stomach 30 minutes after gavage feeding to conscious WT and NPR-A knockout mice. As shown in Figure 3, in wild-type mice, gastric emptying was $87.8 \pm 0.8\%$ for BNP treated (10 ng/g) vs. $97.3 \pm 1.04\%$ for vehicle ($n = 5$, $P < 0.05$, Student's *t* test). For NPR-A KO mice, emptying was $95.8 \pm 0.5\%$ for BNP treated vs. $91.7 \pm 0.7\%$ for vehicle ($n = 5$, $P > 0.05$, Student's *t* test). As shown in Figure 4 this effect on gastric emptying showed a dose dependent relationship with increasing doses of 5, 10 and 100 ng/g of BNP producing a significantly lower rate of gastric emptying ($n = 4$, $P < 0.01$, ANOVA, Fisher's LSD test).

Intestinal transit time was assessed for the eight intestinal segments using the intestinal geometric center of the distribution of the dextran. No significant differences in transit time were found between the treated vs. vehicle groups.

Decrease in Absorption. Absorption measured in relative plasma fluorescence units 1 hour after gavage feeding of 4 kDa FITC-Dextran is depicted in Figure 5. Absorption was significantly lower for BNP treated vs. vehicle in wild-type mice, 98.1 ± 25.9 vs. 28.2 ± 7.8 ($n = 5$ in each group, $P < 0.05$, Student's *t* test). For NPR-A KO mice, there was no significant difference for BNP treated vs. vehicle; 64.4 ± 14.8 vs. 60.6 ± 17.4 ($n = 5$, $P > 0.05$).

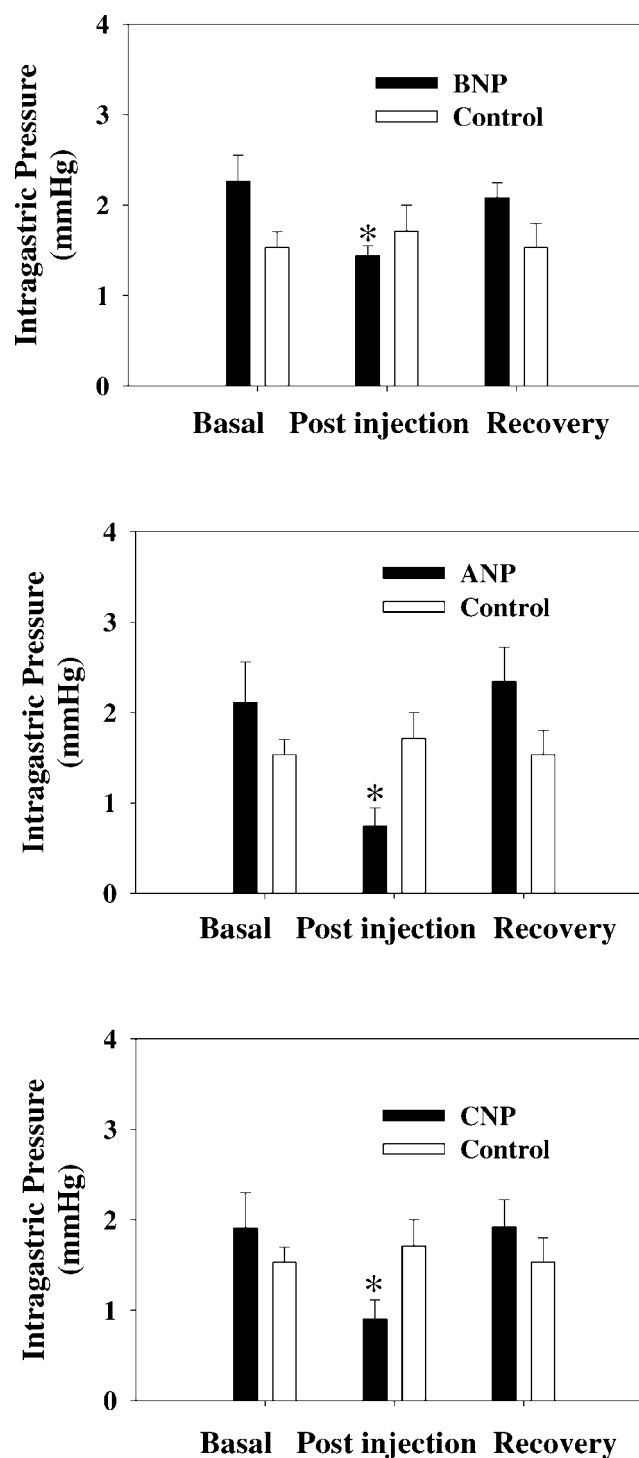


Figure 2. Mean reduction in intragastric pressure (measured in mmHg) following intravenous BNP 10 ng/g in 100 μ l of vehicle vs. 100 μ l of vehicle injection to wild-type (WT) mice ($n = 5$ in each group). Intragastric pressure measured before (Basal), immediately after (Injection) and more than 5 peptide half-lives after injection (Recovery) are shown. BNP, ANP and CNP significantly (*, $P < 0.05$, ANOVA, Fisher's LSD test) decreased intragastric pressure compared to vehicle. Gastric pressure returned toward basal levels when measured later than 5 BNP half-lives.

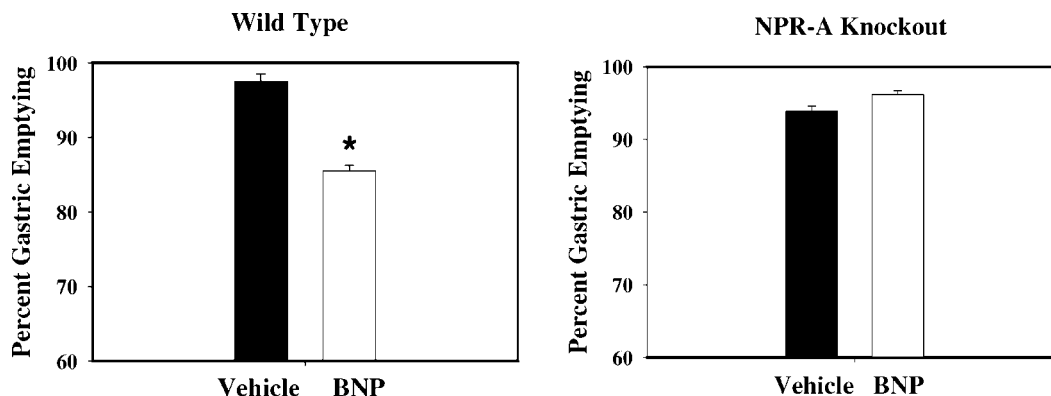


Figure 3. Percent gastric emptying, measured in amount of fluorescence that emptied the stomach as a percentage of the total fluorescence measured in the entire gastrointestinal tract 30 minutes after gavage feeding of 0.01 ml of 0.5 mmol 70 kDa FITC-dextran. BNP (10 ng/g iv) significantly decreased gastric emptying in wild-type mice compared to vehicle ($n=5$, $P < 0.05$, Student's t test). This effect of BNP was absent in NPR-A knockout mice ($n=5$, $P > 0.05$, Student's t test).

Test for Direct Effect of BNP on Plasma Fluorescence. Following iv administration of 0.5 mmol 4kDa FITC-dextran, the relative plasma fluorescence was 237 ± 22.5 in BNP treated vs. 199 ± 22.1 for vehicle treated ($n=4$, $P > 0.05$, Student's t test).

Plasma BNP Levels. BNP levels were measured by radioimmunoassay. Immediately following iv injection of 10 ng/g BNP, plasma BNP levels averaged 4500 pg/ml and the levels fell to 725 pg/ml at 30 minutes post injection and to undetectable levels at 90 minutes post injection.

Discussion

Our experiments show that BNP decreases gastric motility and that this response is accompanied by significant reduction in gastric emptying and gastrointestinal absorption. Prior studies have shown that natriuretic peptides, including BNP, decrease contractility of isolated gastric and intestinal smooth muscle cells *in vitro* (7–9). Our findings clearly demonstrate that this inhibitory effect on the gastrointestinal tract has functional significance in the whole animal. Furthermore, the inhibitory effects of the natriuretic peptides appear to be dose dependent and mediated primarily by NPR-A. As shown in Figure 6, plasma fluorescence 1 hour after iv administration of 4 kDa FITC-dextran was similar in BNP treated vs. vehicle. This finding further strengthens our conclusion that the reduced plasma fluorescence in gavage fed and BNP treated mice was indeed due to decreased absorption or permeability in the GI tract and not due to other known actions of BNP such as increased renal excretion or change in vascular redistribution (16). Although there has been some indication that natriuretic peptides are involved in inhibitory regulation of GI function (17, 18), our findings are the first to document a specific inhibitory role for BNP on emptying and absorptive functions in the gastrointestinal tract.

The average plasma BNP levels immediately after injection averaged 4500 pg/ml. While this level is supra-physiological, it is not uncommon in patients with heart

failure (19). Furthermore, even much higher levels of BNP are consistently seen when recombinant BNP (Nesiritide) is administered for the treatment of heart failure (19, 20). Moreover, the BNP level in our experiments fell to levels routinely seen in heart failure patients at 30 minutes post injection. Therefore, our experimental model provides novel insight into what would be expected in heart failure or when BNP is exogenously administered.

Natriuretic peptides bind to transmembrane receptors that have guanylyl cyclase (GC) activity. ANP and BNP bind to GC-A (NPR-A), whereas CNP binds to GC-B (NPR-B). The ensuing peptide receptor interaction increases intracellular cGMP with subsequent enzymatic steps that regulate cellular functions in various tissues where these peptides and/or receptors are expressed (21).

In our study, NPR-A KO mice did not show a significant response with any of the peptides we used (ANP, BNP, CNP or c-ANP_{4–23}). This suggests that the

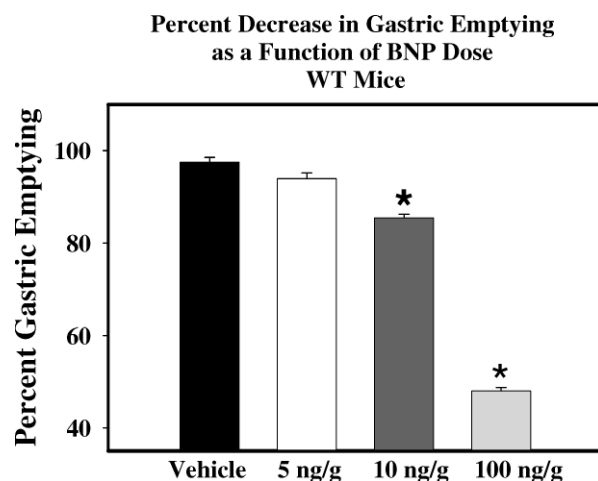


Figure 4. Dose-dependent reduction in percent gastric emptying. Measurements were taken 30 minutes after gavage feeding of 0.01 ml/g of 0.5 mmol FITC-dextran. Progressive doses of BNP at 5, 10 and 100 ng/g iv resulted in significant reduction of gastric emptying. ($P < 0.05$, $n=4$ in each group, ANOVA, Fisher's LSD test).

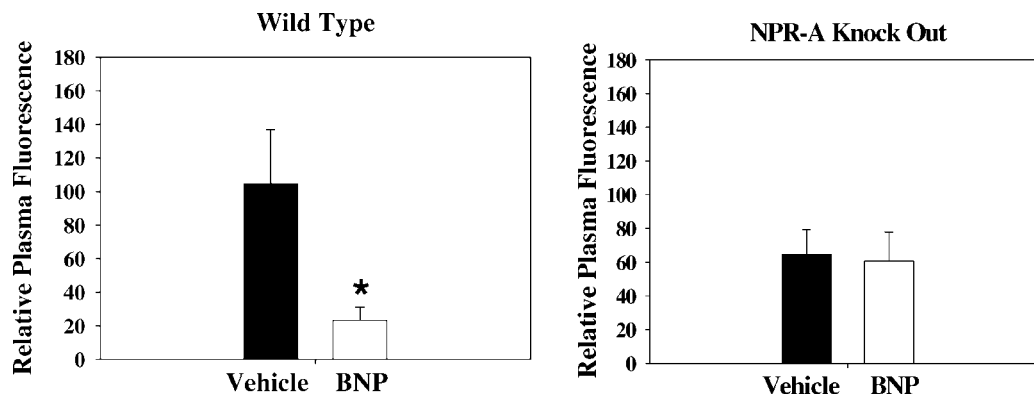


Figure 5. Absorption measured in relative plasma fluorescence units 1 hour after gavage feeding of 4 kDa FITC-dextran in wild-type mice BNP treated (10 ng/g iv) vs. vehicle ($n = 5$, $P < 0.05$, Student's t test). No significant difference in absorption was observed between BNP vs. vehicle treated NPR-A KO mice ($P > 0.05$).

gastrointestinal effects of BNP and the other natriuretic peptides are likely to be specifically mediated by the NPR-A receptor. Since c-ANP₄₋₂₃ specifically binds to NPR-C (22), the absence of a GI effect when c-ANP₄₋₂₃ was injected into WT or NPR-A KO mice is additional evidence that NPR-A may be the major receptor mediating the effect of BNP on gastric emptying and absorption.

Our finding is consistent with previous studies that have shown that the inhibitory effect of CNP on isolated gastric smooth muscle cells is mediated by a cGMP-dependent pathway (23, 24).

The natriuretic peptides in general are among some of the most evolutionarily conserved peptides across many species of the phylogenetic tree with various functions in fluid homeostasis. Studies done early in the discovery of natriuretic peptides have reported that ANP significantly decreased jejunal fluid absorption in dogs and rats (25–28). More recent studies have shown that natriuretic peptides cause upregulation of aquaporin 3 expressions in human colonic epithelia signifying their potential role in fluid

homeostasis (29, 30). ANP has also been shown to be important in promoting sea water adaptation in eels and decreases intestinal sodium absorption (31). However, the great majority of recent studies done on BNP have focused on its role in modulating blood pressure, diuresis and natriuresis. Since the primary stimulus for release of ANP and BNP is mechanical stretch of the atrial and ventricular myocardium (32, 33), their expression and release is closely linked to body fluid volume status (34, 35). Moreover, the temporal pattern of expression and release of BNP following a given stimulus, such as an acute myocardial infarction, indicates that the endocrine heart could potentially employ varying plasma levels of the natriuretic peptides to modulate body fluid volume (36). Since natriuretic peptide receptors are expressed on the gastric and intestinal smooth muscle cells (37, 38), and we show that intravenously administered BNP decreased gastric emptying and absorption, it is logical to deduce that our finding may be an indication that the endocrine heart employs natriuretic peptides to delay or modulate the rate and amount of water and solute absorption from the gastrointestinal tract. From a physiological standpoint the effect of cardiac hormones on absorption in the GI tract is a beneficial extension of their role in volume homeostasis. Theoretically, such a role could extend to pathophysiological states such as heart failure where plasma BNP levels and volume overload progressively rise (32, 39) and modulation of volume status becomes even more critical for survival.

We have shown that high plasma levels of BNP (sustained levels of 500 pg/ml or greater), significantly decrease gastric motility, emptying and absorption in mice. This appears to be a common effect of the natriuretic peptides shared by ANP and CNP. The absence of this GI effect in receptor knockout mice and the dose-response relationship suggests a receptor mediated specific event. While our study in the mouse model cannot be generalized to humans, our findings that BNP significantly decreased gastric emptying and absorption offers valuable new

**Plasma Fluorescence Following Intravenous FITC-dextran
BNP vs. Vehicle, WT Mice**

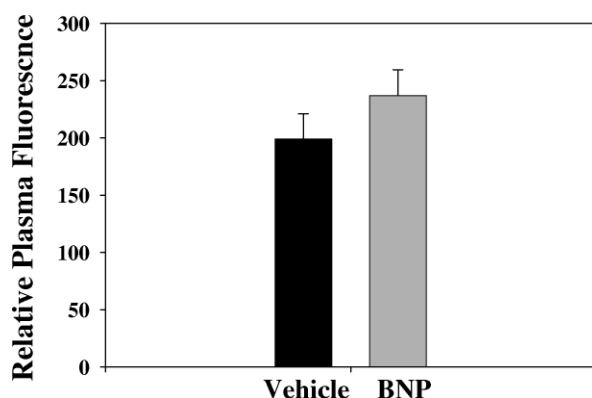


Figure 6. Relative fluorescence of 50 μ l plasma sample taken 1 hour after intravenous administration of 100 μ l 0.5 mmol 4 kDa FITC-dextran. BNP treated (10 ng/g iv) vs. vehicle in wild-type mice, ($P > 0.05$, $n = 4$ in each group, Student's t test).

insights into the role of the gastrointestinal tract in fluid homeostasis, especially during heart failure. First, symptoms of perturbed gastrointestinal function such as nausea, dyspepsia, indigestion and malabsorption are frequently seen in patients with heart failure where plasma BNP levels are markedly elevated (10, 40). Our study suggests that some of these symptoms could at least partly be attributable to the elevated BNP. Secondly, such an effect by BNP could potentially add a new area of interest and investigation in the role of the heart as an endocrine organ. While there are established physiological and pathophysiological cardio-renal regulatory pathways, a possible 'cardio-gastric and/or cardio-intestinal' link via natriuretic peptides appears to be another possible pathway involving the endocrine heart.

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