Kinin B2 Receptors Mediate Blockade of Atrial Natriuretic Peptide Natriuresis Induced by Glucose or Feeding in Fasted Rats

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Abstract—We have shown previously that the kininogen-derived peptides bradykinin, prokinins, and PU-D1, given intravenously or into the duodenal lumen, block the atrial natriuretic peptide (ANP)-induced diuretic-natriuretic effect in fasting, anesthetized rats infused with isotonic glucose. HOE-140, an inhibitor of bradykinin B2 receptors, completely suppresses this ANP blockade. When intravenous glucose infusion is omitted, the above-described inhibition of ANP does not take place. Therefore, to clarify the role of glucose and/or feeding in this phenomenon, we used fasted, anesthetized rats to test how the ANP excretory response was affected by (1) short-term feeding before anesthesia, (2) 1 mL of isotonic glucose introduced into the stomach, and (3) the interaction of HOE-140 with these treatments. In addition, we tested the effects of 1 mL of intragastric glucose administration and HOE-140 on urinary excretion in awake rats. In anesthetized rats, both glucose administration and feeding significantly inhibited the diuretic-natriuretic effect of ANP for up to 90 minutes. Similarly, intragastric glucose delayed spontaneous sodium and water excretion for 90 minutes in awake rats. In all 3 cases, pretreatment with HOE-140 (2.5 μg IV) fully prevented the inhibition of ANP excretory action, ruling out osmotic effects as the cause of reduced diuresis. These results indicate that the presence of glucose in the digestive tract triggers an inhibitory effect on ANP renal actions that requires activation of kinin B2 receptors, providing strong support to our hypothesis that during the early prandial period, gastrointestinal signals elicit a transient blockade of renal excretion with a mechanism involving the kallikrein-kinin system. (Hypertension. 1999;34[part 2]:826-831.)

Key Words: atrial natriuretic factor ■ ANP blockade ■ kininogens ■ peptides ■ bradykinin ■ kinins ■ PU-D1 ■ HOE-140

Bradykinin (BK) and peptides released from kininogen by pepsin, such as prokinins (molecules of 15, 16, and 18 amino acids that share the amino acid sequence of BK) and a 20–amino-acid peptide from domain 1, designated PU-D1, have in common the property of blocking atrial natriuretic peptide (ANP)–mediated diuresis-natriuresis.1–3 This blockade occurs when these peptides are given intravenously or into the duodenal lumen of anesthetized rats, within a narrow range of small subpressor doses, in fasted animals infused intravenously with isotonic glucose solution (IGS). Pretreatment with HOE-140, a specific inhibitor of BK B2 receptors,4 annuls the blockade exerted by these kinins on ANP-mediated diuresis-natriuresis.1,2 PU-D1, a peptide released from kininogen domain 1, has the ability to mimic and reinforce BK as an inhibitor of ANP, but it is 6 to 7 times more active, and its effect lasts longer than BK.3 Remarkably, the inhibitory effects of PU-D1 on ANP are also prevented by HOE-140. On the other hand, neither PU-D1 or BK blocks ANP-mediated diuresis-natriuresis if IGS infusion is omitted in the same type of experiments in fasting rats.5 We proposed that the inhibitory effect of kininogen-derived peptides on ANP excretory action is part of a transient physiological adjustment aimed at compensating for the transference of extracellular fluid into the digestive juices during the initial prandial period. The signaling mechanism would likely involve glucose availability and/or absorption and activation of the kallikrein-kinin system. On the basis of this hypothesis, the present study was undertaken to clarify the role of glucose, or feeding, as well as its relation to endogenous kinins, on the renal excretory response to ANP in anesthetized rats. In addition, we explored the effects of glucose administration and kinin B2 receptors on spontaneous diuresis-natriuresis in awake rats.

Methods
ANP(103–125) (atriopeptin II, rat form) and BK were purchased from Sigma Chemical Co. HOE-140 was a gift from Hoechst, Frankfurt in Main, Germany. Male and female Sprague-Dawley rats (220 to 250 g) were obtained from the university animal facilities. All experimental procedures were performed in accordance with insti-
tutional and international guidelines for welfare of animals, in compliance with the Helsinki Declaration.

Bioassay to Test Anti-ANP Effects in Anesthetized Rats

We used female rats to facilitate cannulation of the urinary bladder, following a similar setup as described previously.1-3 Rats were anesthetized with sodium pentobarbital (45 mg/kg IP) and heparinized. Polyethylene cannulas were introduced in the trachea, femoral, and jugular veins, and a femoral artery. A silastic catheter was placed in the bladder, and urine was collected during 8 successive periods of 20 minutes. Arterial blood pressure was continuously monitored. Three injections of 105 pmol (250 ng) of ANP dissolved in 100 μL of saline were administered intravenously at the beginning of the experiment and the beginning of the first urine-collection period. These time traces in Figures 1 and 2).

Eight experimental groups were used to determine the effects of feeding and intragastric glucose administration, with or without kinin B2 receptor blockade, on ANP-induced urinary excretion. All rats were not allowed to eat for a period of 12 hours overnight (basic fasting condition); thereafter, the different treatments were performed before anesthesia. The time lapse between the end of the experimental maneuver and the beginning of the first urine-collection period was 35 to 40 minutes. The following comparisons were made:

1. Effect of feeding: Fasting control rats (fasted group) were compared with fasting rats allowed to eat ad libitum for a 40-minute period just before anesthesia (fed group).

2. Effect of HOE-140 on feeding: Two groups of rats were treated exactly as above but received 1.92 nmol (2.5 μg) of HOE-140 IV just after cannulation of the femoral vein, ie, 20 minutes before the first urine-collection period. These groups are denoted fasted+HOE and fed+HOE.

3. Effect of glucose: Fasting rats received 1 mL of IGS into the stomach by gavage before anesthesia (glucose group). Similarly, fasting rats received 1 mL of distilled water by the same route as hydration controls (water group).

4. Effect of HOE-140 on glucose: Two groups of rats, treated as above, also received HOE-140 intravenously as in the second group. These groups were denoted glucose+HOE and water+HOE, respectively.

Effect of Glucose and HOE-140 on Spontaneous Diuresis in Awake Rats

Male rats were used to facilitate intravenous injections through the dorsal penis vein. To acclimate the animals to the experimental procedure, rats were administered 1 mL of distilled water by intragastric gavage and placed in individual metabolic cages for 2 hours. This procedure was performed twice, every other day. The third time, the actual experiment was performed. Rats were fasted 12 hours overnight and separated into 4 groups, analogous to the last 4 groups described in the anesthetized-rat protocols, namely, glucose, water, glucose+HOE, and water+HOE. According to the group, animals received either 1.92 nmol (2.5 μg) of HOE-140 or its vehicle (50 μL of saline) intravenously under short-lasting ether anesthesia. Thirty minutes later, the rats were given either 1 mL of IGS or 1 mL of distilled water intragastrically. Rats were placed in the metabolic cages, and urinary excretion was collected at 30, 60, 90, and 120 minutes. In these experiments, the moment of gastric administration was considered time zero.

Analysis of Data

Urinary volume was determined by gravimetry and expressed in mL/kg. Urinary sodium and potassium were measured with an FML3 Flame Photometer (Radiometer) and were expressed in μmol/kg. All values are given as mean±SE. Differences within or between groups were analyzed by Student’s unpaired t test.

Results

In control fasting rats, the first injection of 105 pmol of ANP induced significant increases in urinary sodium (15- to 20-fold), volume (3- to 4-fold), and potassium (2- to 4-fold) excretion compared with noninjected rats1-5 and with the following collection period (Figure 1). The second and third ANP boluses induced successively larger sodium (40- and 120-fold) and volume (5- and 11-fold) excretion, whereas potassium excretory responses remained similar. In contrast, rats allowed to eat for a short period before anesthesia exhibited a striking reduction in sodium and volume excretion in response to the first and second ANP administration (ranging between −77% and −91%). Two hours after the beginning of the experiment, fed rats showed a 50% reduction in volume excretion after the third ANP injection, whereas potassium excretion was larger (88%) than in the fasting group. At the end of the experiment, the weight of the stomach with its content was 2.13±0.29 g in fasting rats and 6.7±0.71 g in rats allowed to eat before anesthesia (P<0.01, unpaired t test). The above-described blockade of ANP-induced urinary sodium and volume excretion in fed rats was completely prevented when the animals were treated with a kinin B2 receptor antagonist before ANP administration (Figure 2). Blockade of kinin receptors per se did not modify the diuretic-saluretic response to ANP in fasted rats (compare traces in Figures 1 and 2).

The ANP blocking effects of recent feeding were closely mimicked by the administration of glucose into the stomach.
(Figure 3). The excretory rates of fasting rats that received 1 mL of distilled water as a hydration control (water group) did not differ from control nonhydrated fasting rats (compare Figures 1 and 3). In contrast, administration of 1 mL of IGS produced a significant inhibition of sodium excretion induced by the first (−288%) and second (−274%) ANP injections and of potassium excretion after the first ANP bolus (−96%; Figure 3). No significant differences in diuresis were observed between groups after any of the 3 ANP injections.

Similar to the case of feeding, kinin B2 receptor blockade annulled the inhibitory effect of IGS administration on ANP-induced sodium and potassium excretion. Moreover, in HOE-140–treated rats, sodium and volume excretion induced by the third ANP injection were larger in the glucose than the water-administered group (62% and 36%, respectively; Figure 4). Treatment with HOE-140 did not modify the ANP responses in hydrated rats (compare traces in Figures 3 and 4).

In addition to inhibiting the renal excretory response to exogenous ANP, intragastric glucose administration significantly delayed spontaneous water, sodium, and potassium excretion in nonanesthetized rats for up to 90 to 120 minutes (Figure 5). No urine output was detected in any group in the first 30 minutes after hydration. After 60 and 90 minutes, rats that received 1 mL of IGS showed a strikingly reduced natriuresis (−71% and −46%), kaliuresis (−52% and −47%), and diuresis (−53% and −36%) compared with rats that received 1 mL of water. Cumulative sodium and water excretion was still significantly lower in IGS-administered rats after 120 minutes (−25% and −15%, respectively). Very interestingly, on a par with the results with exogenous ANP in anesthetized rats, treatment with kinin B2 receptor antagonist fully prevented the antidiuretic effect of glucose administration (Figure 5). In fact, after 120 minutes, HOE-140–treated rats given IGS excreted more sodium (53%) than HOE-140–treated rats given water.

Discussion

The major findings of this study are that short-term feeding or intragastric glucose administration produces a blockade of ANP-induced natriuresis and diuresis that is long lasting (1 to 2 hours) and is mediated by activation of kinin B2 receptors. In addition, glucose administration also blocks spontaneous diuresis-natriuresis in awake rats by an unknown mechanism that also requires functional kinin B2 receptors.

We have demonstrated that ANP-mediated diuresis-natriuresis is strongly inhibited by administration of BK, a
15-amino-acid prokinin, $^2$ PU-D1, $^3$ and pepsin kininogen hydrolysates $^6$ when injected in a narrow range of doses (10 to 200 pmol/rat IV) or in the intestinal cavity in IGS-infused anesthetized rats. The presence of glucose seemed necessary for this inhibition, because neither BK or PU-D1 produced the anti-ANP effect in fasting rats not infused or infused intravenously with isotonic NaCl during the experiment. $^5$

This finding may explain the conflicting differences between our results using BK and HOE-1401 and those found by other authors using BK antagonists in saline-infused anesthetized rats. $^7,^8$ On the other hand, ANP-induced natriuresis-diuresis in isolated perfused rat kidneys is directly inhibited by BK$^9$ or PU-D1 (J.S. Roblero et al, unpublished data, 1999). On the basis of these findings, we proposed that kininogen-derived peptides may be released from the digestive tract and transiently inhibit ANP-induced renal excretion as a physiological mechanism to maintain fluid homeostasis in the early prandial cycle, likely involving glucose as a trigger signal. To test this hypothesis, in the present study we explored the effects of short-term feeding and gastric glucose administration on ANP response in fasted noninfused rats with or without kinin B2 receptor blockade.

We decided to use 3 successive ANP boluses to follow the duration of the inhibitory effect for 3 hours after feeding or IGS gastric administration. The dose of 105 pmol of ANP induces a good excretory response and is not near a saturating range. All drugs were dissolved in saline to avoid any possible interference from delivery of minute amounts of glucose. In these conditions, the natriuretic effect of ANP is blocked for up to 2 hours if the rats are allowed to eat ad libitum for 40 to 60 minutes or when they receive 1 mL of IGS before anesthesia. A less-consistent inhibition pattern was observed with secondary volume and potassium excretion induced by ANP. After feeding, ANP-induced diuresis was inhibited, whereas kaluresis tended to increase; in contrast, in IGS-administered rats, only potassium excretion was transiently reduced, and diuresis was unaffected. These results suggest a specific blockade of ANP effects on sodium transport. In preliminary trials, feeding and gastric IGS administration also blocked, although to a lesser extent, the natriuretic-diuretic action induced by 209 pmol of ANP, the dose used in our previous reports. Because a single injection of 1.92 nmol of HOE-140 completely restored renal excretory response to ANP in identically treated rats, we conclude that the ANP-blocking effect of glucose or feeding is mediated by activation of kinin B2 receptors, presumably by increased levels of endogenous kinins during digestion. We have shown that this dose of HOE-140 blocks the vasodepressor effect of intravenous BK for $>100$ minutes. $^6$ The results with HOE-140 further rule out any significant effect of osmotic changes brought about by food ingestion or glucose administration as determinants of the renal excretion blockade.
Moreover, we explored the physiological relevance of this mechanism in conscious rats not given exogenous ANP. We demonstrated that in these rats, glucose administration reduced spontaneous urinary salt and water excretion for 2 hours compared with rats receiving water, and this effect was not observed after kinin B2 receptor blockade. These results suggest that in the rat, the kinin-mediated inhibitory mechanism described here normally operates and is relevant in the control of diuresis and natriuresis after feeding.

Kinin B2 receptors are involved, as part of the kallikrein-kinin system, in processes such as pain, inflammation, smooth muscle contraction and relaxation, NO production, natriuresis, and blood pressure regulation. It has been indicated that the major function of the renal kallikrein-kinin system is to excrete sodium and water when excess sodium is present in the body.10,11 Surprisingly, according to our present and previous results, in normal rats, the kallikrein-kinin system can mediate a blockade of urinary excretion induced by exogenous ANP in the early part of the prandial period, as well as a blockade of spontaneous sodium excretion after glucose administration. In collaboration with Dr Carlos Vio, we have performed preliminary experiments in kininogen-deficient mutant Brown Norway Katholiek rats12 fed a regular rodent chow and submitted to the same test described here. Those rats respond to exogenous ANP with an excretory response that is blocked by intravenous injection of BK and PU-D1, as do normal rats. However, in these mutant rats, gastric IGS administration does not cause any inhibition of ANP-induced natriuresis-diuresis, and as expected, the ANP response is not affected by HOE-140 (unpublished observations, 1999). These results give strong support to the assumption that the endogenous kallikrein-kinin system plays a sodium-retaining role during the early prandial period.

With the present results, we cannot know at what level kinins exert this modulatory action in the awake or anesthetized rat. The possibility cannot be ruled out that in the prandial period, endogenous kinins contribute to blood flow redistribution toward the intestine and annex glands, facilitating intestinal secretion and indirectly causing renal refractoriness to ANP and/or delaying spontaneous diuresis. However, on the basis of our previous reports, we favored the interpretation of a direct inhibition on the renal effects of ANP. Low doses of BK or kininogen-derived peptides block the excretory action of ANP when given intravenously 3 minutes before the cardiac hormone, without inducing any change in perfusion pressure.1–3 Transient blood flow redistribution away from the kidney is very unlikely under those conditions. In addition, urinary cGMP excretion is also decreased, which indicates a reduced activation of ANP receptors.1 Furthermore, BK blunts the actions of ANP in isolated rat kidneys perfused under constant flow and modulates ANP-induced cGMP production in rat renal medullas in vitro.9

The effect of glucose raised questions about the mechanism of action that allows kininogen-derived peptides to exert the transitory inhibition of ANP. Insulin is an obvious candidate to act as a feeding and/or glucose mediator signal. It is known that hyperglycemia and hyperinsulinemia induce significant reductions in renal sodium and fluid excretion due to enhanced sodium and glucose transport in the proximal tubule that overcomes the elevated glomerular filtration rate observed in those conditions.13–15 Furthermore, there is evidence for an antagonistic interaction between ANP and insulin. The acute administration of r-glucose (300 mg/kg) in normal human volunteers induced a rapid increase in circulating ANP that might counteract the renal sodium-retaining effects of hyperglycemia and hyperinsulinemia.16 Exogenous insulin does not increase ANP levels, but low-dose ANP infusion counteracts the sodium-retaining action of insulin.17 Although we have not explored the participation of insulin in our studies, our present results suggest that the negative interaction between this hormone and ANP may require activation of kinin B2 receptors. This idea warrants further investigation.

We may speculate that the inhibition of the renal excretory action of ANP in the early prandial period is necessary to fulfill the secretory demand of the digestive tract glands. ANP is a vasodilator and also facilitates microvascular fluid escape to the extravascular space.18–20 In both these processes, kinins might act synergistically with ANP to promote net fluid secretion to the intestinal lumen. In the same line of thought, it is possible that in the early prandial period, activation of digestive proteolytic enzymes (kallikrein, pepsin, or other enzymes) releases peptides from kininogen, such as BK and PU-D1, which at precise low concentrations block renal excretion. These interactions open a wide field for exploration.

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References


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