Neurokinin A increases duodenal mucosal permeability, bicarbonate secretion, and fluid output in the rat

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Hälgren, Anneli, Gunnar Flemström, Per M. Hellström, Mikael Lördal, Sandra Hellgren, and Olof Nylander. Neurokinin A increases duodenal mucosal permeability, bicarbonate secretion, and fluid output in the rat. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1077–G1086, 1997.—The aim of this study was to examine the integrative response to neurokinin A (NKA) on duodenal mucosal permeability, bicarbonate secretion, fluid flux, and motility in an in situ perfusion model in anesthetized rats. Intravenous infusion of NKA (100, 200, and 400 pmol·kg−1·min−1) induced duodenal motility. Furthermore, duodenal mucosal bicarbonate secretion, fluid output, and mucosal permeability increased in response to NKA. Pretreatment with the nicotinic antagonist hexamethonium did not change the response in any of the parameters investigated, whereas the NK3-receptor antagonist MEN 10,627 effectively inhibited all responses to NKA. Indomethacin induced duodenal motility and stimulated bicarbonate secretion. In indomethacin-treated rats, NKA further increased motility but decreased indomethacin-stimulated bicarbonate secretion by 70%. The NKA-induced increase in mucosal permeability was unaltered by indomethacin. It is concluded that NKA not only induces motility but also increases mucosal permeability and fluid output. Furthermore, the neuropeptide may have both stimulative and inhibitory effects on bicarbonate secretion. All responses to NKA are dependent on NK-2 receptor activation but are not mediated through nicotinic receptors.

duodenum; motility; neurokinin-2 receptor antagonist; tachykinin; cyclooxygenase inhibitor

Recent studies (8) have shown that the duodenal mucosa in a variety of mammalian species, including humans, secretes bicarbonate into the viscoelastic mucous gel adherent to the underlying epithelium. It has been proposed that the resulting juxtaepithelial "mucus-bicarbonate barrier" forms the major line of defense against acid-induced mucosal injury (1). Various physiological mechanisms influence and regulate duodenal mucosal bicarbonate secretion (8). For instance, recent experiments (10, 18, 19) strongly suggest that elevation of intraluminal pressure, either by distension or induction of duodenal motility, increases the secretion of bicarbonate. Our hypothesis is that the elevated intraluminal pressure activates mechanoreceptors and, via a neural reflex arc, stimulates the bicarbonate-secreting duodenocytes.

The tachykinin neurokinin A (NKA) is involved in the control of gastrointestinal motility in several species. It has been suggested to be the dominant and most potent tachykinin in stimulating rat duodenal contractions (11, 13, 15, 17), either by acting directly on the smooth muscle cells or by indirect mechanisms mediated by acetylcholine release (6). One of the aims of this study was to test whether NKA also stimulates duodenal alkaline secretion.

Almost all tachykinins in the gut occur in two types of neurons: intrinsic and extrinsic. The tachykinin-containing intrinsic neurons form a dense network in the myenteric plexus and project to circular but not longitudinal muscle (21). At least three different subsets of tachykinin receptors, denoted NK1, NK2, and NK3, have been identified (4, 15). Although NKA binds to all three receptor types, it has by far the highest affinity for NK2 receptors. NK2 binding sites have been localized both in the circular muscle layer and, to a lesser extent, in the longitudinal muscle layer of rat duodenum (3, 5, 9).

Neuropeptides of the tachykinin family, released from afferent sensory neurons, can induce and sustain neurogenic inflammation (20). The alterations in the tissues associated with this release might also affect intestinal mucosal permeability. The second aim of this study was therefore to determine whether the proinflammatory mediator NKA influences mucosal permeability. The surgical procedure was similar to that previously described by Nylander et al. (16) (though the kidneys were retained intact and only the duodenum was cannulated). In brief, we performed tracheotomies on the rats to facilitate spontaneous breathing and inserted a PE-90 cannula containing heparin (12.5 IU/ml dissolved in isotonic saline) into the left common carotid artery for blood sampling. The right femoral artery was catheterized and connected to a pressure transducer (Gould P23 ID, Gould Electronics, Bilthoven, Netherlands) and a polyrecorder (Polygraph model 7D, Grass Instruments, Quincy, MA) to monitor arterial blood pressure.

Methods

Surgical Procedures

Male Sprague-Dawley rats (Møllegaard Breeding Center, Ejby, Denmark), weighing 200–300 g, were deprived of food but given free access to drinking water for 18 h before the experiments. The animals, kept in groups of two or more, were maintained under constant conditions (12:12-h light-dark cycle; temperature, 21°C). The rats were anesthetized with 120 mg/kg body wt Na-5-ethyl-1-(1-methyl-propyl)-2-thiobarbituric acid (Inactin) administered intraperitoneally. The surgical procedure was similar to that previously described by Nylander et al. (16) (though the kidneys were retained intact and only the duodenum was cannulated). In brief, we performed tracheotomies on the rats to facilitate spontaneous breathing and inserted a PE-90 cannula containing heparin (12.5 IU/ml dissolved in isotonic saline) into the left common carotid artery for blood sampling. The right femoral artery was catheterized and connected to a pressure transducer (Gould P23 ID, Gould Electronics, Bilthoven, Netherlands) and a polyrecorder (Polygraph model 7D, Grass Instruments, Quincy, MA) to monitor arterial blood pressure.
Both femoral veins, and in some experiments also the jugular vein, were catheterized for infusion of $^{51}$Cr-EDTA and the administration of drugs.

The abdominal cavity was opened by a midline incision, and a PE-10 catheter was inserted into the common bile duct $\sim 2$ mm from its entrance into the duodenum to prevent pancreaticobiliary secretions from entering the duodenal segment. A soft plastic tubing (1 mm ID; Silastic, Dow Corning) was introduced via the mouth and gently pushed through the esophagus into the stomach and then through the pylorus into the duodenum. This tubing was secured by a ligature 2–4 mm distal to the pylorus. Another cannula (PE-320) was inserted into the duodenum through an incision $\sim 3$ cm distal to the pylorus and secured by ligatures. The orally introduced tubing was connected to a peristaltic pump (Gilson Minipuls 3, Villiers, Le Bel, France) for perfusion of the duodenal segment at a constant rate. The abdominal cavity was stitched closed, and the body temperature of the animal was maintained at $\sim 37.5^\circ$C by means of a heating pad controlled by an intrarectal thermistor. All experiments were approved by the Uppsala Ethics Committee for Animal Experiments.

**Bicarbonate Secretion**

The rate of luminal alkalinization was determined by titration of the effluent to pH 6 with 50 mM HCl, under continuous gassing with 100% N$_2$, using pH-stat equipment (Autoburette ABU 12, TTT 80 Titrator, and PHM 64 pH meter, Radiometer, Copenhagen, Denmark). The pH electrode was routinely calibrated with standard buffers before titration. The rate of luminal alkalinization was expressed as the net amount of base (micromoles) secreted per centimeter of intestine per hour.

**Mucoal Permeability**

After completion of surgery, we administered the radioactive isotope $^{51}$Cr-EDTA intravenously as a bolus of $\sim 75$ µCi followed by a continuous infusion at a rate of 50 µCi/h (adapted to obtain a constant plasma level of $^{51}$Cr-EDTA). The isotope was diluted in a Ringer-bicarbonate solution, infused at a rate of 1 ml/h (infusion pump from Harvard Apparatus, Edenbridge, UK). One hour was permitted for tissue equilibration of the $^{51}$Cr-EDTA and for the animal to recover from surgery. Three to four blood samples (0.2 ml) were collected at regular intervals during the experiment, and the blood volume loss was compensated for by the injection of a 5% Ficoll solution. After centrifugation of the samples, we removed 50 µl of the plasma for measurement of radioactivity. The luminal perfusate and the blood plasma were analyzed for $^{51}$Cr activity (gamma counter 1282, Compuamma CS, Pharmacia, Uppsala, Sweden). A linear regression analysis of the plasma samples was made to obtain a corresponding plasma value for each effluent sample. The clearance of $^{51}$Cr-EDTA from blood to lumen was calculated according to the following formula

$$^{51}\text{Cr-EDTA clearance} = \frac{\text{effluent (cpm/ml) \times perfusion rate (ml/min)}}{\text{plasma (cpm/ml) \times tissue weight (g)}} \times 100 \text{ g}$$

$^{51}$Cr-EDTA clearance was expressed as milliliters per minute per 100 g wet tissue weight. The values given in the text are mean increases, calculated by subtracting the mean basal permeability (i.e., before NKA administration) from each value obtained during NKA infusion ($\Sigma$ (sample clearance – mean basal clearance)/6).

**Fluid Secretion and/or Absorption**

Vials were weighed individually, before and after effluent collection, on an electronic precision balance (Sauter AR 1014, Albstadt-Ebingen, Germany). The effluent weight was thus determined as the weight difference for each vial. The mean weight of effluent collected during the control period (the first 30 min or, in some of the protocols, the first 20 min of each experiment) was subtracted from the weight of each subsequent effluent. The difference was expressed as grams of fluid per gram of wet tissue weight per hour. The technique used provides information about relative changes of fluid flux across the duodenal mucosa, and a positive fluid flux value indicates increased secretion or decreased absorption.

**Motility**

Duodenal contractions (motility) were monitored as changes in intraluminal pressure. A pressure transducer (Gould P23 ID) was connected to the inlet cannula of the perfusion system via a T-tube. Changes in intraluminal pressure were recorded on the Grass polyrecorder (see above). Duodenal motility was assessed by calculating the fraction of time (in percent) occupied by contractions, i.e., the fractional contraction time (FCT).

**Experimental Protocols**

In all groups, the duodenal segment under study was perfused with saline at a constant rate of 0.4 ml/min both during the hour of recovery and subsequently throughout the experiment. During the experiment, the effluent was collected in 10-min samples. All experiments began with a control period to assess basal conditions.

Infusion of 100, 200, or 400 pmol·kg$^{-1}$·min$^{-1}$ NKA. After a 30-min control period, NKA was infused for 60 min in a volume of 1 ml/h. The experiments continued an additional 30 min after the infusion was stopped. NKA was administered in three different doses: 100 pmol·kg$^{-1}$·min$^{-1}$ (n = 5), 200 pmol·kg$^{-1}$·min$^{-1}$ (n = 5), and 400 pmol·kg$^{-1}$·min$^{-1}$ (n = 6).

Infusion of 400 pmol·kg$^{-1}$·min$^{-1}$ NKA in animals treated with hexamethonium. An additional group of animals (n = 5) was treated with the nicotinic receptor antagonist hexamethonium intravenously; 20 mg/kg was given as a bolus 15 min before the start of effluent collection and immediately followed by 10 mg·kg$^{-1}$·h$^{-1}$ as a continuous infusion throughout the experiment. This group received NKA (400 pmol·kg$^{-1}$·min$^{-1}$) for 60 min, and the experiments were terminated after an additional 30-min period, as described above.

Intermittent infusion of NKA. In this group of rats (n = 5), NKA was administered as an intravenous infusion at a rate of 400 pmol·kg$^{-1}$·min$^{-1}$ for 5 min. The infusion pump was then turned off for 5 min. This procedure was repeated throughout the 60-min infusion period. Following an additional 30 min after the end of the intermittent infusion, the experiments were terminated.

Pretreatment with MEN 10,627. Thirty minutes before the start of effluent collection, an intravenous infusion of the NK-2 receptor antagonist MEN 10,627 was started. The infusion began with a bolus of 0.5 µmol/kg MEN 10,627 infused for 10 min, immediately followed by 0.5 µmol·kg$^{-1}$·h$^{-1}$ MEN 10,627 throughout the experiment (n = 6). MEN 10,627 was dissolved in dimethyl sulfoxide (DMSO), and the infused volume was kept very low (total infused volume <150 µl/kg). A 100-µl Hamilton glass syringe (Hamilton Bonaduz, Bonaduz, Switzerland) was used. After a 30-min control period, NKA (400 pmol·kg$^{-1}$·min$^{-1}$) was infused intravenously for 60 min.
Vehicle controls. This group of animals (n = 4) first received the same volume of the vehicle (DMSO) instead of MEN 10,627 and subsequently received NKA as described above.

Pretreatment with indomethacin. Twenty minutes after the start of effluent collection, the animals (n = 7) were given an intravenous bolus of indomethacin (5 mg/kg). After an additional 40-min period, NKA (400 pmol·kg⁻¹·min⁻¹) was infused intravenously for 30 min. The experiments were continued an additional 30 min after cessation of the NKA infusion.

Indomethacin controls. This group of animals (n = 6) received indomethacin (5 mg/kg) in the same manner as described above, i.e., after a 20-min control period, and served as controls to the indomethacin-NKA group.

Controls. One group of rats (n = 6) served as time controls (120 min) and did not receive any of the tested drugs.

Chemicals

Inactin was obtained from Research Biochemicals (Natick, MA). NKA was purchased from Peninsula Laboratories (Mereseyside, UK). MEN 10,627 was a kind gift from Dr. A. C. Maggi (Menarini Pharmaceuticals, Florence, Italy). Indomethacin (Confortid for injection) was obtained from Dumex (Copenhagen, Denmark). Ficoll, hexamethonium chloride, and NaCl were all purchased from Sigma Chemical (St. Louis, MO). Heparin was from Pharmacia and EDTA from NEN-Du Pont (Boston, MA).

Statistics

Values are expressed as means ± SE. The statistical significance of data was tested by analysis of variance with contrast (Fisher protected least-significant difference test) by comparing results before and after drug treatment (repeated measures) and by comparing differences between groups of animals (nonrepeated measures). Student’s t-test was also used where appropriate. The values given in RESULTS are pooled from all the measurements taken during the basal or infusion period. All statistical analyses were done on a Macintosh computer using Statview software (Abacus Concepts, Berkeley, CA). P < 0.05 was considered significant (two-tailed test).

RESULTS

Effects of NKA

All doses of NKA significantly increased bicarbonate secretion (Fig. 1). The mean increase in bicarbonate output did not differ among the three doses tested (3.7 ± 0.6, 2.9 ± 0.6, and 2.4 ± 0.6 µmol·cm⁻¹·h⁻¹ for 100, 200, and 400 pmol·kg⁻¹·min⁻¹ NKA, respectively), although the tendency was that of decreasing secretion with increasing doses of NKA. Furthermore, in the group receiving the highest dose of NKA, secretion declined with time during the infusion period, and during the last 20 min of infusion the rate of bicarbonate secretion was not significantly different from basal secretion. After cessation of the infusion, regardless of the dose of NKA used, bicarbonate secretion returned to basal level and did not differ from that in control animals.

All doses of NKA increased fluid output (Fig. 1), and there was no difference in mean fluid output between the groups (2.2 ± 0.2, 2.6 ± 0.2, and 1.8 ± 0.3 g·g⁻¹·h⁻¹ for 100, 200, and 400 pmol·kg⁻¹·min⁻¹ NKA, respec-
tively). After cessation of the NKA infusion, fluid output was similar to that under control conditions or, in the case of the highest dose of NKA, slightly negative during the first 10 min immediately after the infusion ($P < 0.05$).

Calculations of the amount of base (bicarbonate) in the net fluid secreted showed that the concentration of bicarbonate was significantly lower ($P < 0.05$) than in interstitial fluid (assumed to be 24 mM in calculations) at 200 and 400 pmol·kg$^{-1}$·min$^{-1}$ (13 ± 3 and 14 ± 3 mM, respectively) but not significantly different at 100 pmol·kg$^{-1}$·min$^{-1}$ (17 ± 4 mM).

During basal conditions, as well as in control animals, spontaneous contractions were very rare. Infusion of NKA induced duodenal motility in a dose-dependent manner (Fig. 1). The mean FCT was 21.2 ± 1.5%, 46.9 ± 9.2%, and 72.4 ± 4.3% for 100, 200, and 400 pmol·kg$^{-1}$·min$^{-1}$, respectively, and there were significant differences among the effects of all doses. As depicted in Fig. 2, the NKA-induced motility was very intense, especially at the two higher doses, and disappeared only minutes after termination of the infusion.

All doses of NKA increased mucosal permeability (Fig. 3). However, the increase was more profound and sustained at the highest dose. The mean increase during NKA infusion were 0.18 ± 0.04, 0.23 ± 0.04, and 0.45 ± 0.08 ml·min$^{-1}$·100 g$^{-1}$ for 100, 200, and 400 pmol·kg$^{-1}$·min$^{-1}$, respectively (Fig. 3). The mean increase at 400 pmol·kg$^{-1}$·min$^{-1}$ was significantly higher than in the groups infused with 100 or 200 pmol·kg$^{-1}$·min$^{-1}$ ($P < 0.05$).

Infusion of NKA did not have any sustained effect on mean arterial blood pressure (in some animals a transient dip was observed) compared with controls (data not shown).

Effects of NKA in Animals Treated with Hexamethonium

After hexamethonium, NKA (400 pmol·kg$^{-1}$·min$^{-1}$) increased bicarbonate secretion, mucosal permeability, and fluid output and induced duodenal motility in a manner similar to that in animals receiving NKA alone (Figs. 4 and 5). Although mucosal permeability appears to be slightly elevated in hexamethionium-treated animals compared with the group receiving NKA alone, there was no significant difference in mean increase between the groups. Mean arterial blood pressure was lower in animals treated with hexamethonium (63 ± 2 mmHg) than in controls (101 ± 5 mmHg; $P < 0.01$).

Intermittent Infusion of NKA

To investigate the possible involvement of adaptation in the response to NKA, an intermittent infusion of
NKA (400 pmol·kg$^{-1}$·min$^{-1}$) was given in 5-min intervals over 1 h. The protocol thus results in an average dose, calculated for the entire infusion period, of 200 pmol·kg$^{-1}$·min$^{-1}$. This group of animals was therefore compared with the groups receiving either 200 or 400 pmol·kg$^{-1}$·min$^{-1}$ as a continuous infusion. Intermittently infused NKA increased the rate of alkalinization (Fig. 6), and the mean increase (3.1 ± 0.4 µmol·cm$^{-2}$·h$^{-1}$) did not differ from either 200 or 400 pmol·kg$^{-1}$·min$^{-1}$. The fluid output was also similar to that in continuously infused rats (Fig. 6).

Because of the rapid offset of the effect of NKA on duodenal motility, duodenal contractions disappeared for some time between NKA infusion periods (Fig. 2). The motility pattern was similar to that obtained in response to indomethacin (see below). FCT was approximately one-half (33.6 ± 5.4%) of that in animals receiving 400 pmol·kg$^{-1}$·min$^{-1}$ NKA as a continuous infusion (Fig. 6). There was no significant difference in mean FCT, calculated from the entire 60-min infusion period, between intermittent infusion and continuous infusion of 200 pmol·kg$^{-1}$·min$^{-1}$ NKA. However, at steady state, which was considered to be the last 30 min of the infusion, there were differences in mean FCT between all three groups.

Finally, intermittent infusion of NKA also increased mucosal permeability (Fig. 7). The mean increase (0.27 ± 0.03 ml·min$^{-1}$·100 g$^{-1}$) was similar to that of 200 pmol·kg$^{-1}$·min$^{-1}$ NKA infused continuously but differed significantly from that of 400 pmol·kg$^{-1}$·min$^{-1}$ NKA ($P < 0.01$).

Effects of NKA in Animals Treated with MEN 10,627

Neither the NK-2 receptor antagonist MEN 10,627 ($n = 6$; data not shown) nor the vehicle DMSO ($n = 4$;
data not shown) had any effect on basal conditions. However, MEN 10,627 efficiently inhibited the responses to NKA (400 pmol·kg⁻¹·min⁻¹) in all parameters investigated (Figs. 4 and 5). In contrast to the group receiving NKA alone, the group treated with the antagonist did not exhibit any changes in net bicarbonate output, fluid flux, or mucosal permeability in response to NKA.

In some experiments, motility, although sparse and with low amplitude, occurred toward the end of the NKA infusion. Thus the mean FCT was slightly but significantly increased (4 ± 2%). However, compared with the motility observed in animals receiving NKA alone (72 ± 4%), the motility was still very low (Fig. 4).

Animals infused with the vehicle (DMSO) responded to NKA in a manner similar to that described for NKA given alone (n = 4; data not shown).

Effects of Indomethacin

We have previously shown that the cyclooxygenase inhibitor indomethacin induces duodenal motility and increases alkaline secretion (19). The purpose of the following experiments was to compare the response to NKA to that induced by indomethacin. The rate of alkalinization was stimulated by indomethacin (Fig. 8). The mean increase, calculated from the first 60 min after administration, was 6.2 ± 0.7 µmol·cm⁻¹·h⁻¹, which is significantly higher than that obtained during 60 min infusion of either dose of NKA. Administration of indomethacin increased fluid output transiently, and this increase was significant only during the first 10 min (Fig. 8). Indomethacin also induced duodenal motility with a mean FCT of 31.2 ± 2.6% (Fig. 8). However, the motility pattern differed from that obtained in rats receiving a continuous infusion of NKA as regular periods of profound motility were alternated with quiescent intervals. Indomethacin slightly and transiently (the first 10 min after administration) increased mucosal permeability (Fig. 9). Finally, mean arterial pressure was slightly decreased (by 10 mmHg) in rats treated with indomethacin (data not shown).

Effects of NKA in Animals Treated with Indomethacin

The following experiments were performed to evaluate whether NKA affects duodenal mucosal alkaline secretion in animals subjected to prostaglandin synthesis blockade and thus with ongoing motility. In indomethacin-treated animals, NKA decreased the rate of alkalinization and during the last 20 min of infusion the bicarbonate secretion did not differ from basal secretion (Fig. 8). The net rate of secretion during steady state, i.e., the 20 min immediately before the start of NKA infusion and the last 20 min of NKA infusion, was 3.6 ± 0.3 and 1.0 ± 0.4 µmol·cm⁻¹·h⁻¹, respectively. Thus NKA decreased indomethacin-stimulated bicarbonate secretion by 72% (P < 0.001). Fluid output was only slightly increased by NKA, and the mean increase during 30 min of NKA infusion was lower than in animals receiving NKA alone (0.8 ± 0.3 g·g⁻¹·h⁻¹ in indomethacin-treated animals compared with 2.6 ± 0.6 g·g⁻¹·h⁻¹ in animals receiving NKA alone; P < 0.01).

Fig. 6. Effects of intermittent infusion of NKA (400 pmol·kg⁻¹·min⁻¹) on 3 of the investigated parameters. We alternated 5-min periods of NKA infusion with 5-min periods without infusion (Δ; n = 5). The other 2 groups received NKA as a continuous intravenous infusion for 60 min in 2 different doses [200 pmol·kg⁻¹·min⁻¹ (○; n = 5) and 400 pmol·kg⁻¹·min⁻¹ (●; n = 6)]. Arrows indicate times of commencement and cessation of intermittent or continuous NKA infusion. A: effect of NKA on net rate of duodenal mucosal alkaline secretion. B: effect of NKA on fluid flux relative to basal values. C: effect of NKA on duodenal motility assessed as FCT occupied by contractions.
In indomethacin-treated rats, infusion of NKA increased FCT from 33.0 ± 3.9% to 72.6 ± 4.2% (p < 0.001; Fig. 8). Thus NKA induced motility of the same magnitude whether or not the rats were treated with the cyclooxygenase inhibitor. NKA infusion increased mucosal permeability in indomethacin-treated animals (0.47 ± 0.10 ml·min⁻¹·100 g⁻¹) to a similar extent as in rats receiving NKA alone (0.52 ± 0.1 ml·min⁻¹·100 g⁻¹; Fig. 9).

**DISCUSSION**

The present study demonstrates that the tachykinin NKA, apart from being a potent stimulant of duodenal motility, also increases bicarbonate secretion, mucosal permeability, and fluid output in the duodenum. Pre-treatment with MEN 10,627, a potent and highly selective antagonist to NK-2 receptors (14), did not influence basal parameters but inhibited all effects otherwise induced by NKA treatment. The involvement of NK-2 receptors in all observed responses is thus indicated.

We have previously suggested a relationship between duodenal motility and mucosal secretion of bicarbonate. The hypothesis is based on the following findings: 1) elevation of intraluminal hydrostatic pressure by distension increases duodenal mucosal bicarbonate secretion (19); 2) basal duodenal mucosal alkaline secretion is twice as high in the few rats exhibiting spontaneous duodenal contractions compared with those that do not (18); 3) cyclooxygenase inhibitors, such as indomethacin, as well as the nitric oxide (NO) synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME), induce duodenal motility and increase bicarbonate secretion concomitantly (10, 19). It has also been shown (10, 19) that distension-, indomethacin-, and L-NAME-induced increases in duodenal mucosal bicarbonate secretion are abolished by hexamethonium. In contrast, the present study demonstrates that both the NKA-induced motility and the increase in duodenal mucosal bicarbonate are independent of nicotinic transmission and thus differ from the responses obtained in indomethacin- or L-NAME-treated rats.

It has recently been shown that indomethacin, despite an additional increase in duodenal motility, does not further increase L-NAME-induced bicarbonate secretion. This implies that the assumed reflex arc is already maximally activated (18). In theory, if NKA affected bicarbonate secretion solely by activation of the postulated reflex arc, the neuropeptide would not further increase indomethacin-stimulated alkaline secretion. In fact, NKA actually decreased indomethacin-stimulated bicarbonate secretion by more than 70%. This finding strongly suggests the existence of an inhibitory mechanism triggered by the neuropeptide. Two additional findings further substantiate the involvement of an inhibitory component in the response to NKA. First, despite the profound duodenal motility induced by NKA, the increase in alkalization was considerably smaller than that obtained in indomethacin-treated rats. Second, bicarbonate secretion declined with time, especially at the highest dose of NKA.

Although the presence of an inhibitory component in the NKA-induced response is clear, the net effect of NKA was that of increased bicarbonate secretion. The mechanism involved is probably not related to the...
induction of motility for the following reasons: 1) the time courses of motility and secretion differ; 2) there is a dose-response relationship in the effect of NKA on motility, whereas bicarbonate secretion is unaltered with increasing dose; 3) as mentioned above, the mechanism is insensitive to hexamethonium and thereby differs from that of L-NAME-, indomethacin-, and distension-induced secretion (10, 19). Should the hypothesis of a causal relationship between duodenal motility and mucosal bicarbonate secretion be rejected based on the presented data? To reject the hypothesis, it has to be assumed that NKA, when given systemically, has only one mode of action, i.e., induction of duodenal motility. This assumption is contradicted by the fact that NKA diminished indomethacin-stimulated alkalinization. Therefore, we speculate that NKA activates the reflex arc by virtue of its stimulative effect on duodenal motility and concomitantly inhibits signal transmission to the enterocytes (see Fig. 10).

An alternate explanation for the increase in alkaline secretion in response to NKA must be sought. Based on the effect of NKA in indomethacin-treated rats, the stimulative action of the neuropeptide may be attributed to the release of endogenous prostaglandins. Prostaglandin E2, prostaglandin E2.

Fig. 8. Effects of indomethacin (Indo) alone (5 mg·kg⁻¹·h⁻¹; □; n = 6) and NKA (400 pmol·kg⁻¹·min⁻¹) in Indo-treated rats (●; n = 7) on 3 of the investigated parameters. A: net rate of duodenal mucosal alkaline secretion in response to Indo alone and to NKA in Indo-pretreated rats. B: effect of NKA in Indo-treated animals and Indo alone on fluid flux. C: effect of Indo alone and NKA in Indo-treated animals on duodenal motility.

Fig. 9. Effect of Indo alone (□; n = 6) and NKA (400 pmol·kg⁻¹·min⁻¹) in Indo-treated rats (●; n = 7) on mucosal permeability expressed as net change from basal permeability.

Fig. 10. A schematic model of the assumed reflex arc and the proposed effects of NKA on it. NKA activates the reflex arc by virtue of the stimulative action on duodenal motility. However, NKA concomitantly inhibits signal transmission from the postulated mechanoreceptor to the enterocyte. Instead, the stimulative action of NKA on bicarbonate secretion may, at least in part, be attributed to release of endogenous prostaglandins. The involvement of paracellular transport of bicarbonate cannot be excluded, but does not fully explain the increase in alkalinization in response to the tachykinin. PGE₂, prostaglandin E₂.
taglaidin E₂, which is a well-known stimulant of duodenal mucosal bicarbonate secretion (8), is released in response to NK-2 receptor activation (7).

Because NKA also increases paracellular permeability, the possibility of a parallel increase in diffusion of bicarbonate ions has to be considered. However, whereas the increase in ⁵¹Cr-EDTA clearance in response to NKA was further augmented by the highest dose of NKA, the net secretion of bicarbonate tended to decrease with increasing dose of the tachykinin. Furthermore, the net secretion during NKA infusion was lower in indomethacin-treated animals, despite the maintained increase in mucosal permeability, than in rats receiving NKA alone. Thus an increased paracellular transport of bicarbonate ions does not fully explain the increased rate of alkalinization in response to NKA. The exact mechanism by which NKA increases duodenal bicarbonate secretion remains unclear.

Infusion of NKA increased the blood-to-lumen clearance of ⁵¹Cr-EDTA, suggesting an increased paracellular permeability across the duodenal epithelium. Interestingly, the permeability of the tight junctions can be altered by pressure and/or volume changes in the intercellular space (2). Moreover, NKA increases the permeation of Evans blue, indicative of an increased vascular permeability to macromolecules, in the duodenum (12). We speculate that an increased filtration of vascular fluid and plasma proteins in response to NKA elevates interstitial fluid pressure. Subsequently, the high interstitial pressure affects the size and shape of the epithelial paracellular pathways and thereby increases the pore area for diffusion of ⁵¹Cr-EDTA across the mucosa.

NKA increased the secretion (or decreased the absorption) of fluid in the duodenum. Furthermore, the concentration of bicarbonate in the net fluid output was lower than in the interstitial fluid, suggesting that NKA stimulates the secretion of another ion, possibly chloride (22). The NKA-induced increase in fluid flux is not dependent on nicotinic transmission but may involve endogenous prostaglandins since indomethacin diminished this response.

It is concluded that NKA, apart from being a potent stimulant of duodenal motility, also increases duodenal epithelial permeability to ⁵¹Cr-EDTA and the output of fluid. NKA also increases duodenal mucosal bicarbonate secretion. However, since the indomethacin-induced increase in bicarbonate secretion was attenuated by NKA, dual effects of the neuropeptide are suggested; a dominant stimulative action and a weaker inhibitory action. The stimulative action is probably not related to the induction of motility but could be due to the release of endogenous prostaglandins. Nicotinic receptors are not involved in mediating any of the investigated effects, whereas the activation of NK-2 receptors is essential to all the responses obtained in response to NKA.

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