Pharmacological analysis of components of the change in transmural potential difference evoked by distension of rat proximal small intestine in vivo

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Larsson MH, Sapnara M, Thomas EA, Bornstein JC, Lindström E, Svensson DJ, Sjövall H. Pharmacological analysis of components of the change in transmural potential difference evoked by distension of rat proximal small intestine in vivo. Am J Physiol Gastrointest Liver Physiol 294: G165–G173, 2008. First published November 1, 2007; doi:10.1152/ajpgi.00264.2007.—The reflex response to distension of the small intestine in vivo is complex and not well understood. The aim of this study was to characterize the neural mechanisms contributing to the complex time course of the intestinal secretory response to distension. Transmucosal potential difference (PD) was used as a marker for mucosal chloride secretion, which reflects the activity of the secretomotor neurons. Graded distensions (5, 10, and 20 mmHg) of distal rat duodenum with saline for 5 min induced a biphasic PD response with an initial peak (rapid response) followed by a plateau (sustained response). The rapid response was significantly reduced by the neural blockers tetrodotoxin and lidocaine (given serosally) and by intravenous (iv) administration of the ganglionic blocker hexamethonium and the NK1 receptor antagonist SR-140333. Serosal TTX and iv SR-140333 significantly reduced the sustained response, which was also reduced by the NK1 receptor antagonist talnetant and by the vasoactive intestinal polypeptide (VPAC) receptor antagonist [4Cl-o-Phe6, Leu17]-VIP. Serosal lidocaine and iv hexamethonium had no significant effect on this component. Inhibition of nitric oxide synthase had no effect on any of the components of the PD response to distension. The PD response to distension thus seems to consist of two components, a rapidly adapting and adapting component operating via nicotinic transmission and NK2 receptors, and a slow component operating via VIP-ergic transmission and involving both NK3 and NK2 receptors.

mouse; intestinal mucosa; chloride secretion; tachykinin receptors; VIP; nitric oxide; VPAC receptors

INTESTINAL MOTOR ACTIVITY and mucosal secretion interact in a highly complex fashion that is not well understood. In the fasting state, they both change cyclically in a rhythm that is called the migrating motor complex (MMC) (39). In a recent modeling study, we proposed that the MMC is generated via a recurrent network of myenteric sensory neurons that alternate between maximal firing and no activity due to the interplay of positive feedback and activity-dependent depression (59). Submucous neurons are directly or indirectly mechanosensitive (23, 27, 60), and myenteric neurons can be activated by stretch (29). It therefore seems likely that the intense motor activity that occurs during maximal firing of the sensory networks may trigger the secretory component in response to phase III of the MMC rhythm. The time course of this component is, however, not easily explained by a simple cause-and-effect relationship (28). Interestingly, MMC phase III-evoked intestinal secretion is increased in celiac disease (39) and in a substantial subgroup of patients with irritable bowel syndrome (32). Thus a better understanding of the mechanisms involved in this reflex has pathophysiological relevance.

The reflex pathways involved in distension-evoked intestinal secretion are complex. In experimental animals, mechanical stimulation, such as mucosal stroking and distension of the intestine, initiates luminal chloride secretion (7, 23, 27, 39). However, the exact mechanisms are not well understood. Weber et al. (60) proposed that both extrinsic and intrinsic primary afferents are involved in distension-induced secretion, whereas Cooke et al. (12) concluded that, at least in response to mucosal stroking, intrinsic primary afferents alone mediate the secretory response. Several studies have indicated that intestinal chloride secretion from the mucosa is mainly controlled by the submucous plexus (26, 51), but it was recently shown that mucosal stimulation can activate secretomotor neurons also via long myenteric pathways (46). In addition, Kunze et al. (29, 30) have reported that myenteric intrinsic primary afferent neurons are excited by mechanical stimuli like stretch. The complexity of the response may therefore well reflect involvement of both myenteric and submucous neural mechanisms.

Recently we studied the effects of cholera toxin on the duodenal secretory response to distension in vivo. We observed that nicotinic receptor blockade with hexamethonium did not have any significant effect on the mean potential difference (PD) response but appeared to change its time course with a relative inhibition of the initial response (28; S. Kordasti and H. Sjövall, unpublished observations). This suggests that alternative neural mechanisms mediating the secretory response to distension might be distinguished pharmacologically, thereby identifying some elements of these pathways. In the present study, we pursued this by analyzing in more detail the neurotransmission mechanisms responsible for the rapid and slow components. Tachykinin antagonists were...
used to block sensory transmission, serosal lidocaine was used to anesthetize the myenteric plexus, hexamethonium was used to block nicotinic transmission, the nitric oxide synthase (NOS) inhibitor Nω-nitro-l-arginine (l-NNA) was used to test the involvement of the nitric oxide (NO) system, atropine was used to block muscarinic transmission, and a vasoactive intestinal peptide (VIP) receptor antagonist was used to block vasoactive intestinal polypeptide (VPAC) receptors.

MATERIALS AND METHODS

Animals

The study was performed in male Sprague-Dawley rats (Möllegard, Ejby, Denmark or B&K Universal, Uppsala, Sweden), weighing 300–400 g, and male C57BL/6 mice, weighing 20–25 g (bred in-house). The animals were housed in groups of at least two in plastic cages with free access to water and food. The cages were placed in a temperature-controlled environment (19–23°C) with humidity of 25–70% and a light/dark cycle of 12 h. All the experiments were approved by the Animal Ethics Review Committee in Göteborg, Sweden.

Surgical Preparation and Experimental Setup

Preparations for duodenal distensions in rats. The general experimental setup and surgery procedure have been described previously (56). Briefly, after an overnight fast, anesthesia was induced by using pentobarbital sodium (60 mg/kg ip) and was maintained with infusion of chloralose (3.6 mg/ml ia), which also contained glucose (138 mM) and NaHCO₃ (33 mM) to prevent dehydration and acidosis. The right femoral artery and vein were prepared to continuously monitor arterial pressure and for the administration of drugs. The abdomen was opened via a midline incision, and the duodenum and the pancreatico-biliary duct were localized. A 5- to 6-cm-long duodenal segment distal to the duct, containing an artery large enough to supply the segment with blood after ligation, was identified. The segment was isolated between ligatures, and the remaining intestine was put back into the abdominal cavity. The luminal contents were flushed gently with warm (37°C) saline. A double lumen catheter was inserted into the proximal end of the segment. The catheter contained one polyethylene tube (PE 90) for fluid administration and pressure measurement via a pressure transducer (DPT-6000 Single-use transducer; Peter von Berg Medizintechnik, Engelharting, Germany) and one polyethylene tube (PE 240) filled with saline-agar (4%) for transmucosal PD recordings. The agar bridge was immersed in a beaker containing 1 M KCl and a calomel half cell (REF 401; Radiometer, Copenhagen, Denmark). Another agar bridge was placed in the abdominal cavity, and its other end was immersed in 1 M KCl containing a similar calomel half cell. The distal end of the intestinal segment was cannulated with a plastic tube, which was clamped during distensions and kept open between distensions. A ground reference electrode was placed in the femoralis cavity, and a thermometer was placed into the rectum to monitor body temperature. The reference electrode was placed in the femoralis cavity, and a thermometer was placed into the rectum to monitor body temperature. The pressure transducers used for recording systemic blood pressure and intestinal luminal pressure were connected to bridge amplifiers on a Grass polygraph (model 7D; Grass Instruments, Quincy, MA), and the signals were collected in digital form by using a Labview program (National Instruments, Austin, TX). The calomel half cells were connected to a high-impedance bridge amplifier (DVC 1000 Current/voltage clamp; World Precision Instruments, Sarasota, FL). These signals were sampled at 4 Hz with the Labview program.

Preparations for colonic distensions in rats. In some experiments, we studied the response to colonic distension in rats. These rats were prepared in the same way as those used in the duodenal experiments, except that a different segment was used. The colonic segment was 5–6 cm long and taken starting ~2 cm distal to the caecum.

Preparations for duodenal distensions in mice. We also studied the PD response in mouse distal duodenum. Mice were prepared in the same way as rats, except that they were anesthetized by inhalation of isoflurane (Forene, Abbot Scandinavia AB). In this group, blood pressure was not monitored. The duodenal segment in mice was 3–4 cm. The proximal double lumen catheter used for fluid administration and pressure recording (PE 50) and PD recording (PE 160) were smaller.

Experimental Protocol

After the abdominal surgery, PD and intraluminal pressure were recorded in the isolated intestinal segment. The segment was allowed to recover for at least 15 min while a stable baseline PD was established. Thereafter, the distal end of the segment was clamped, and a bolus of saline was injected intraluminally into the closed segment until the desired pressure level was reached. If necessary, pressure was adjusted manually by adding or removing saline. At the end of the distension period (usually 5 min), fluid was emptied from the intestinal segment by opening the distal clamp. A small amount of fluid was always left in the segment to maintain a PD signal. In most experiments, distensions lasted 5 min and were separated by 10-min intervals. This protocol was chosen on the basis of the slow and complex time course of the PD response to MMC phase III in humans (32, 39). In a few experiments, we used a different protocol, with distensions lasting 30 s instead of 5 min. This protocol was used to delineate the mechanisms behind the complex time course of sustained distensions. The segment was distended at 5, 10, and 20 mmHg, i.e., at pressures within the physiological range.

Drugs and Chemicals

The contribution of neurons operating via voltage-gated sodium channels was tested by serosal application of tetrodotoxin. Lidocaine, a poorly diffusible local anesthetic agent, was given serosally to preferentially block the myenteric plexus. The involvement of tachykinin receptors 1, 2, and 3 was evaluated using appropriate antagonists, and the role of feedback from intestinal smooth muscle was studied by giving atropine, the muscarinic receptor antagonist. The role of fast nicotinic transmission, the most probable mode of transmission between the two plexuses, was evaluated by administering the nicotinic receptor antagonist hexamethonium. A VIP receptor blocker (the VPAC antagonist [4Cl-d-Phe⁶, Leu⁵⁷]-VIP) was given to block neural and/or epithelial VPAC receptors, and granisetron was used to determine the role of released 5-HT operating via 5-HT₃ receptors. The involvement of NO in this system was tested with the NOS inhibitor l-NNA.

The doses and routes of delivery of the drugs were chosen to match doses shown to be effective in earlier studies in the literature. TTX, lidocaine, hexamethonium (8), and granisetron (53) all reduce cholera toxin-induced secretion at the doses used in the present study. SR-140333, SR-48968 (33), and atropine (56) reduce colonic motility at the relevant doses, whereas the VPAC receptor antagonist [4Cl-d-Phe⁶, Leu⁵⁷]-VIP has been shown to reduce distension-induced secretion (28). The NOS inhibitor l-NNA, potentiates the permeability response to luminal bile acids and enhances motility and systemic pressure (57). TTX was purchased from Alomone labs (Jerusalem, Israel). The tachykinin NK₁ receptor antagonist (SR-140333), the tachykinin NK₂ receptor antagonist (SR-48968), and the tachykinin NK₂ receptor antagonist talnetant (SB-223412) were synthesized at AstraZeneca R&D Mölndal, Sweden. Hexamethonium, atropine, [4Cl-d-Phe⁶, Leu⁵⁷]-VIP, and lidocaine (Na⁺-channel blocker) were obtained from...
Sigma-Aldrich (Stockholm, Sweden). Granisetron (Kytril, Roche) (5-HT3 receptor antagonist) was purchased from a retail supplier. L-NNA was obtained from Sigma-Aldrich. All drugs were dissolved in isotonic saline, except for the NK receptor antagonists. SR-140333 and SR-48968 were dissolved in 5% ethanol, 5% solutol (obtained from BASF, Göteborg, Sweden), and 90% isotonic saline. SB-223412 was given as a nanosuspension (34). The compounds were administered intravenously at the start of the experiment, i.e., before the start of the distension protocol, unless otherwise described.

Data Analysis

All raw data were stored as ASCII files on a personal computer. The data processing was done in MatLab (Release 14SP3; The Mathworks, Lowell, MA), with the use of custom-made software. The increase in systemic arterial pressure was calculated as the mean value during distension minus the mean value during the last 5 min preceding distension.

The calculation of the PD was performed in two ways. First, the overall shape of the PD response was determined for each experiment. Each curve for each experiment in its respective group was coordinated exactly in time, and a mean curve was calculated for each group. Secondly, the details of the individual PD curves were calculated. In this analysis, the beginning and end of each distension were marked as trigger points, and the PD data 5 min prior to, during, and 5 min after each distension were extracted and further analyzed. The following parameters were calculated (see Fig. 1B): 1) the PD at the onset of distension (baseline PD, mV), 2) the maximum PD during distension (maximal PD, mV), 3) the PD at the end of distension (PD end, mV), and 4) the rate of rise (i.e., the slope) of the initial PD increase (mV/s).

The MatLab program plotted the original curve and suggested values for the different parameters; these values could also be adjusted manually to deal with errors due to noise in the signal. Adjustments were rarely necessary and were done by a person unaware of the interventions. The baseline PD value was consistently subtracted from peak and end PD values, since it can be argued that a shift in baseline might account for altered absolute levels. As reported previously, the PD curve generated by distension was biphasic (28), with an initial peak followed by adaptation to a lower plateau. The two components are referred to as rapid and sustained responses. The rapid response was defined as a local maximum within the first 2 min, minus baseline PD. The sustained response was defined as PD at the end of distension, minus baseline PD. Please note that in all figures, the polarity of the PD signal is reversed, i.e., higher (positive) plotted PD values denote a more lumen-negative PD.

Statistics

A large group of untreated rats (n = 26) was used as controls for all interventions. The occurrence of a group difference at each pressure was tested through a standard linear repeated measure model with autoregressive heteroscedasticity (1) within subject correlation structure by using t contrasts. The occurrence of group differences of the cumulative values was tested with an ANOVA (heteroscedasticity) by using t-contrast. A P value less than 0.05 was regarded as statistically significant. There was no adjustment for multiple testing. The data are expressed as mean values ± SE.

RESULTS

Shape and Time Course of the Control Response

Luminal distension of the duodenum for 5 min induced a biphasic PD response, consisting of an initial PD increase leading to a transient PD peak (defined as the rapid response) (Fig. 1B). After the rapid response, PD levels decreased and reached a steady elevation lasting until the end of the distension period (referred to as the sustained response) (Fig. 1B). There was no significant difference between PD levels at 4 and 5 min after onset of distension, indicating the level did, indeed, stabilize. Distending the duodenum with increasing pressure

![Fig. 1. Changes in the transmucosal potential difference (PD) (A, bottom) and systemic arterial blood pressure (A, top) when using 5-min and 30-s distension paradigms in distal rat duodenum. The average value from 26 (5-min distension) and 8 (30-s distension) rats are shown. A magnification of the PD response at 20 mmHg and the parameters used in the subsequent data analysis are shown in 5-min distension (B) and 30-s distension (C).](http://ajpgi.physiology.org/)
(5, 10, and 20 mmHg) demonstrated that both the rapid and sustained responses were pressure dependent (Fig. 2, A–C).

To evaluate the consequences of changing the duration of the stimulus, the response to the standard sustained distension (5 min) was compared with that of a short-lasting (30 s) distension. Reducing the duration of the pressure increase from 5 min to 30 s did not affect the amplitude or the rate of rise of the rapid response at any pressure applied (Fig. 1A). The sustained response was not seen after 30-s distensions; instead the response to the standard sustained distension after the PD decreased and eventually returned to baseline levels after ~5 min at all pressures applied (Fig. 1, A and C). The time to reach baseline levels was similar with the use of 5-min distensions or 30-s distensions.

To evaluate to what extent the PD response to distension was species and/or segment specific, we compared its time course in rat and mouse duodenum and in rat colon. The PD response to distension in mouse duodenum was biphasic with a similar pressure-dependent shape to that in rats (Fig. 2, A–C). However, baseline PD and the distension-evoked rapid and sustained responses were significantly elevated in mice compared with rats (Fig. 2, A–C, P < 0.05). The rate of rise was pressure dependent in mice and almost fivefold more rapid at pressures of 10 and 20 mmHg than in rats (Fig. 2D).

In rats, colonic distension induced a PD response with a similar time course to that evoked by duodenal distension. However, colonic baseline PD was markedly elevated compared with duodenal baseline PD (Fig. 3A). In the colon, both the rapid and the sustained responses were pressure dependent and significantly larger than in the duodenum at all distension pressures tested (Fig. 3, B and C, P < 0.01). The rate of rise of the PD response was roughly twofold higher in the colon than the duodenum at pressures of 10 and 20 mmHg (Fig. 3D, P < 0.01).

Effects of Serosal Tetrodotoxin and Lidocaine

TTX. The involvement of voltage-gated sodium channels was tested by serosal application of the Na⁺-channel blocker TTX. This has previously been shown to block a major part of the PD response to intestinal distension in vitro (23, 27, 60). In vivo, TTX cannot be given intravenously in sufficient doses to accomplish neural blockade without causing toxic systemic effects. TTX was therefore given serosally at the highest dose that did not lead to any severe changes in systemic pressure (28).

TTX (0.1–0.2 ml, 3 μg/ml, applied 5 min before each distension) did not affect the initial baseline PD before the first distension but lowered baseline PD about 25% between subsequent distensions (Fig. 4A). TTX inhibited the rapid response at 5 mmHg and significantly reduced the rapid response to higher pressures compared with nontreated controls (Fig. 4, A and B, P < 0.01). However, the rate of rise of the remaining rapid response (Fig. 4D) was not significantly affected by TTX.

The sustained response was abolished by TTX at 5 mmHg and was reduced by ~40% at pressures of 10 and 20 mmHg (Fig. 4, A and C, P < 0.01).

Lidocaine. Lidocaine diffuses slowly through the tissue and, with this mode of administration, does not reach the mucosa itself (8). Lidocaine (0.5 mg/10 cm intestine, locally applied to the serosa every tenth minute) significantly reduced the amplitude of the rapid response but had no significant effect on the sustained response (Fig. 4, B and C). It also significantly reduced the rate of rise of the rapid response (Fig. 4D, P < 0.05) but did not significantly affect baseline PD (data not shown).

Role of Tachykinin Networks: Effects of the Tachykinin Receptor Antagonists

Tachykinin receptors play a key role in transmission in both the myenteric and submucous plexus, but the role of each receptor subtype (NK₁, NK₂, and NK₃) is unclear. The effects of antagonists for all three types of receptors on the time course of the distension response were therefore tested. The selective tachykinin NK₁ receptor antagonist SR-140333 (3 μmol/kg iv) significantly reduced the baseline PD before the onset of distension (P < 0.05). Neither the tachykinin NK₂ receptor antagonist (SR-48968) (3 μmol/kg iv) nor the NK₃ receptor antagonist talnetant (SB-223412) (3 μmol/kg iv) affected baseline PD (Fig. 5A). SR-140333 significantly reduced the rapid...
and sustained responses by about 30% (P < 0.01) and 20% 
(P < 0.05), respectively (Fig. 5, B and C). The sustained
response was reduced by talnetant (P < 0.05) but was unaf-
fected by SR-48968 (Fig. 5C). All three NK receptor antagonists reduced the rate of rise of the
rapid response, although significance was only reached with
SR-140333 and SR-48968 (Fig. 5D, P < 0.05).

Role of Extrinsic Efferent Neurons and/or Intramural Fast 
Transmission: Effects of Nicotinic Receptor Blockade

Hexamethonium blocks fast nicotinic transmission in both
peripheral and enteric ganglia (40). Treatment with hexame-
thonium (10 mg/kg, bolus dose iv, given at the start of the
experiment and repeated after 45 min) reduced the rapid
response (P < 0.05) but had no effect on the sustained
response (Fig. 6, A and B). The rate of rise of the rapid
response was also markedly reduced by hexamethonium (Fig. 
6C, P < 0.05). Hexamethonium did not affect baseline PD
(data not shown).

Effects of VIP Receptor Blockade and Inhibition
of NO Production

[4Cl-d-Phe^6, Leu^17]-VIP (infusion at a rate of 2 
µg·kg^{-1}·min^{-1}) significantly reduced the sustained response but had no significant effect on the amplitude of the rapid
response (Fig. 6, A and B) or its rate of rise (Fig. 6C). The
baseline PD tended to be reduced, but this effect was not
statistically significant (data not shown).

The NOS inhibitor L-NNA [bolus dose of 10 mg/kg followed
after 10 min by an iv infusion of 3 mg/kg (57)] significantly
increased systemic pressure but had no significant effect on
baseline PD (data not shown), the pressure-induced rapid and
sustained PD responses, or the rate of rise of the rapid response (Fig. 6. A-C).

Effects of Muscarinic Receptor Blockade

Muscarinic receptors contribute to secretomotor control by
opening Ca^{2+}-gated potassium channels in enterocytes, which
generate a short-lasting PD increase (16, 17). Muscarinic
receptors also play a major role in excitation of smooth muscle.
We therefore tested the effect of atropine on both components

Fig. 3. Comparison between distension-evoked PD signals in rat duodenum 
(n = 26) and rat colon (n = 8). The intestinal segments were distended to 5, 
10, and 20 mmHg. A: average values of the raw data tracings from the entire 
control groups. B–D: calculated values representing the rapid (B) and sustained 
(C) responses to distension, as well as the rate of rise of the rapid response (D). 
Data are presented as means ± SE. **P < 0.01 duodenum compared with 
colon at respective pressure.

Fig. 4. Effects of TTX (0.1–0.2 ml, 3 
µg/ml, locally applied to the serosa 5 
min before each distension) and lidocaine (0.5 mg/10 cm intestine, locally 
applied to the serosa every 10th minute). A: average value of the raw data 
tracings from the entire TTX (n = 7) and control (n = 26) groups. B–D: 
cumulative values (5, 10, and 20 mmHg have been added together) for the 
rapid (B) and sustained (C) responses to distension, as well as the average 
value (5, 10, and 20 mmHg have been averaged) for the rate of rise of the rapid 
response (D). Data are presented as means ± SE. *P < 0.05, **P < 0.01 
compared with control experiments.
of the PD response to distension. Rats treated with atropine (0.5 mg/kg iv) had similar rapid and sustained PD responses to controls at all pressures applied, both with regard to amplitude and rate of rise of the rapid response (data not shown).

Effects of 5-HT3 Receptor Blockade

Serotonin released from enterochromaffin cells induces intestinal secretion via 5-HT3 receptors, an effect that is reduced by granisetron (45, 53). We therefore tested the effect of this antagonist on the time course of the distension response.

Granisetron (selective 5-HT3 receptor antagonist, 40 
\text{ng/kg iv}) had no significant effect on either the rapid or the sustained response, except for an increase of the rapid response at 20 mmHg (5.3 ± 0.9 vs. 3.7 ± 0.3 mV, granisetron and controls, respectively, \( P < 0.01 \)). It did not affect the rate of rise of the rapid response.

DISCUSSION

The present set of experiments extends the previously published finding (28) that distension of distal rat duodenum in vivo induces a complex PD response, which is biphasic with a rapid and a sustained component, and is at least partially neurally mediated. Both the rapid and sustained components were partially blocked to a similar extent with serosal TTX and intravenous SR-140333, a NK1 receptor antagonist. The rapid component was reduced by the nicotinic antagonist hexamethonium and by serosal lidocaine, whereas these compounds had no effect on the sustained response. [4Cl-D-Phe6, Leu17]VIP, a VPAC receptor antagonist, and talnetant, a NK3 receptor antagonist, left the rapid component unaffected but reduced the sustained component. The PD response to distension thus seems to involve two distinct components, a rapid and a sustained phase, mediated by different neural mechanisms.

Both the rapid and sustained responses were reduced \( \sim 40\% \) by TTX, thus implying that both responses are at least partially neurally mediated. These effects are in agreement with the PD response to distension. Rats treated with atropine (0.5 mg/kg iv) had similar rapid and sustained PD responses to controls at all pressures applied, both with regard to amplitude and rate of rise of the rapid response (data not shown).

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### Figures

**Fig. 5.** Effects of the tachykinin antagonists SR-140333 (NK3, 3 \text{μmol/kg}), SR-48968 (NK2, 3 \text{μmol/kg}), and SB-223412 (NK1, 3 \text{μmol/kg, nanosuspension}) on baseline PD before distensions (A), on the distension-induced rapid (B) and sustained (C) responses, and on the rate of rise of the rapid response (D). The duodenum was distended to 5, 10, and 20 mmHg, and cumulative values of pressures 5, 10, and 20 mmHg are presented (A–C). D: mean values are presented. Data are presented as means ± SE. \(* P < 0.05\), \(** P < 0.01\) compared with control experiments.

**Fig. 6.** Effects of the tachykinin antagonists SR-140333 (NK3, 3 \text{μmol/kg}), SR-48968 (NK2, 3 \text{μmol/kg}), and SB-223412 (NK1, 3 \text{μmol/kg, nanosuspension}) on baseline PD before distensions (A), on the distension-induced rapid (B) and sustained (C) responses, and on the rate of rise of the rapid response (D). The duodenum was distended to 5, 10, and 20 mmHg, and cumulative values of pressures 5, 10, and 20 mmHg are presented (A–C). D: mean values are presented. Data are presented as means ± SE. \(* P < 0.05\), \(** P < 0.01\) compared with control experiments.
with data obtained by Frieling et al. (23) in guinea pig colon. TTX-sensitive currents dominate in most enteric neurons, but TTX-resistant currents have been demonstrated in AH/Dogiel type II neurons (48), which may thus be active in the presence of TTX. TTX should, however, block action potentials in secretomotor effector neurons. It is unclear if the partial effect of TTX was dose related, since it was impossible to give higher doses due to systemic toxicity. It can therefore, by no means, be excluded that a component of the distension response was mediated via an unknown nonneural mechanism.

The similarities between the time course of the in vivo and the previously described in vitro responses suggest that a substantial component of the response is mediated by intrinsic neurons. This interpretation is also supported by the isolated effect of hexamethonium, the ganglionic-blocking agent, on the initial component of the response. There is, however, an extensive intramural network of sensory neurons: the AH/Dogiel type II neurons that are found in both the myenteric and submucous plexus (24, 44). These neurons are synaptically connected to each other via slow excitatory synaptic potentials (EPSPs), i.e., via connections that are hexamethonium resistant and form interconnected networks that can be self-reinforcing because of recurrent excitatory transmission (2, 58). Myenteric AH/Dogiel type II neurons respond to stretch (30), whereas submucosal AH/Dogiel type II neurons probably respond to mucosal distortion (44) [for a review see Furness et al. (24)].

Myenteric neurons can excite submucous secretomotor neurons via nicotinic receptors (40). In the present study, hexamethonium reduced both the magnitude and the rate of rise of the rapid response but had no effect on the sustained response. Interestingly, hexamethonium did not block the distension-induced secretory response in studies performed in vitro with only the submucous plexus present (23, 52), consistent with a role of nicotinic transmission as a mediator of the connection between the two plexuses. Likewise, our results demonstrated that local application of lidocaine [applied on the serosal side to locally block the myenteric plexus (8)] reduced the magnitude and the rate of rise of the rapid response without affecting the sustained response, effects which are very similar to those produced by hexamethonium. Hence, one may speculate that the myenteric plexus is more involved in the control of the rapid response.

The tachykinin substance P (SP) is found in a large number of enteric neurons, including myenteric and submucosal AH/Dogiel type II neurons (3, 24, 35, 40). SP is recognized as the preferred ligand for NK1 receptors, although it has appreciable affinity for NK2 and NK3 receptors (37). NK1 and NK3 receptor immunoreactivity are colocalized in both myenteric and submucosal Dogiel type II neurons of the rat small intestine (25, 38, 55). Earlier studies have shown that endogenous tachykinins such as SP evoke secretion via both NK1 and NK3 receptors (22, 36), and a combination of NK1 and NK3 receptor antagonists attenuates distension-evoked secretion in guinea pig ileum in vitro (60). However, the contribution of each receptor subtype to distension-evoked secretion was not evaluated in that study.

In the present study, the selective NK1 receptor antagonist SR-140333 reduced both the rapid and sustained PD responses to distension, whereas talnetant, a selective NK3 receptor antagonist, reduced the sustained response only. SR-140333 also significantly reduced the rate of rise of the rapid response. The effect of SR-140333 on the rapid component was very similar to the effect with hexamethonium and serosal lidocaine. It is therefore tempting to speculate that this component was due to an NK1 receptor circuit in the myenteric plexus that connected to secretomotor neurons via a nicotinic transmission step. There are, however, several other possible models that can account for this pattern. One possibility is that a combination of NK1 receptor and nicotinic input is required for the circuit to activate rapidly and that blockade of either component will prolong the time taken to activate the circuit and reduce final firing rate. A pattern of this type was described in previous modeling work on the submucous plexus (9). Serosal lidocaine might, in that case, affect both components.

In contrast to the rapid response, the sustained response was unaffected by hexamethonium and lidocaine but was reduced by TTX, by NK1 and NK3 receptor blockade, and by the VPAC receptor antagonist [4Cl-d-Phe6, Leu17]-VIP. The pharmacology of [4Cl-d-Phe6, Leu17]-VIP is confusing and somewhat contradictory. In a previous study (28), the same antagonist given at the same dose blocked a cholera toxin-induced PD increase and also blocked the PD response to a systemic VIP infusion. On the other hand, the same antagonist did not block VIP-mediated secretion in isolated intestine in the Ussing chamber (15). Some of these paradoxes may be related to network behavior of VIP neurons and relative distributions and affinities of epithelial and neural VPAC receptors. Sensory AH/Dogiel type II neurons have been shown to communicate with VIP-containing S neurons via fast EPSPs (54), whereas the VIP-containing submucous neurons communicate and backproject to each other via slow transmission (47). When the VIP neurons pass through adjacent ganglia, they have been shown to have axonal varicosities and occasional varicose collaterals (19). VPAC receptors may also function as autoreceptors, thus facilitating the release of VIP (49). The most likely explanation for the preferential effect of [4Cl-d-Phe6, Leu17]-VIP on the sustained response is that it is due to activation of VIP neuron networks, with [4Cl-d-Phe6, Leu17]-VIP acting by preventing recurrent feedback. Better VPAC receptor antagonists are clearly needed to more carefully evaluate the mode of action of this peptide antagonist.

The negative results with the muscarinic antagonist atropine were somewhat surprising, but this observation is not unique to our system. Schulzke et al. (50) was likewise unable to demonstrate involvement of muscarinic receptors in distension-induced secretion in the rat colon, whereas Itasaka et al. (27) demonstrated a 20% reduction of the response to distension in rat colon by atropine. Moreover, muscarinic slow synaptic transmission was not detected in electrophysiological recordings from cells in the myenteric plexus of rat duodenum (4) and colon (5). In contrast, several studies in the guinea pig, with the use of both electrical field stimulation and distension to induce secretion, have revealed muscarinic components of the respective responses (10, 11, 23, 31). Part of the discrepancy may be species dependent, since 50% of S-Type I neurons and 20% of AH neurons generate slow muscarinic depolarizations in guinea pigs (41, 42). The absence of any major effect of atropine also argues against any major role of feedback from smooth muscle in the response.

An alternative mechanosensory system is release of 5-HT from enterochromaffin cells. However, although distension has been reported to release mucosal 5-HT (6), it has recently been
shown that this depends on the distension-evoking peristaltic contractions (1). Analysis of the contractile responses produced by the distensions used in this present study indicates that they do not produce peristaltic contractions (28). Thus it is unlikely that the distension stimuli used in this study released mucosal 5-HT. This is consistent with our finding that gastrin-releasing peptide (a 5-HT3 antagonist) did not affect the rapid or sustained responses and is in agreement with the in vitro studies performed by Frieling et al. (23) and Engelmann et al. (18), who did not show any effect with a 5-HT3 receptor antagonist. On the other hand, it has been reported that 5-HT may be relatively more important in activating neural pathways when secretion is induced by mucosal distortion (13, 14), a stimulus that does induce release of 5-HT from the mucosa (20). In rat small intestine, the PD effect of luminal 5-HT is blocked by a 5-HT3 antagonist but not by a 5-HT4 antagonist (21), indicating that any mucosal release of 5-HT in the present study would be expected to act via 5-HT3 receptors.

Finally, there are some comments on the differences in the time course between rats and mice. The rate of rise of the rapid response in the duodenum was 4–6 times faster in mice than in rats. Nurgali et al. (43) have shown that in mouse distal colon, neurons with Dogiel type II morphology often do not exhibit a pronounced after-hyperpolarizing potential (AHP). Interestingly, in recent modeling studies (9, 59), we have shown that suppression of the AHP and/or increased synaptic efficacy in submucous sensory networks will lead to enhanced responses of VIP secretomotor neurons to sensory stimulation (9). Thus a potential explanation for the increased response in mice could be due to a reduced AHP in AH neurons, rendering them more sensitive to, for instance, mechanical stimuli.

In summary, the PD response to distension at physiological pressures (5–20 mmHg) has two components: an initial rapid phase requiring nicotinic transmission and NK1 receptors, sensitive to serosal lidocaine, and a slower sustained phase, resistant to hexamethonium and serosal lidocaine but sensitive to VIP and NK1 antagonists. We previously reported that cholera toxin modifies this response via a mechanism involving 5-HT3, VIP receptors, and prostaglandins (28). Pharmacological analysis of the interactions between these two systems should make it possible to more clearly define the site of action of cholera toxin on the neural circuitry.

REFERRENCES