**ABSTRACT**

Substance P is generally considered an excitatory neurotransmitter related to gut motor activity, although an inhibitory influence of neurokinin-1 (NK1) receptor activation on peristalsis has also been reported. With an optimized in vitro method to assess distention-induced peristalsis, we aimed to clarify the effect of NK1 receptor activation on peristaltic activity and to reveal the mechanisms by which NK1 activation alters peristalsis. Distention of the small intestine of the mouse and guinea pig induced periodic occurrence of rhythmic waves of propagating rings of circular muscle contraction, associated with slow waves and superimposed action potentials, that propelled intestinal contents aborally. Activation of NK1 receptors by Ava[-Pro9,N-MeLeu10] substance P(7-11) (GR 73632) and Sar9, Met(O2)11 on smooth muscle cells resulted in prolongation of the activity periods and increased action potential generation occurring superimposed on the intestinal slow wave activity. Activation of NK1 receptors on interstitial cells of Cajal resulted in an increase in slow wave frequency. Slow wave amplitude increased, likely by increased cell-to-cell coupling. The NK1 antagonist (S)-1-[2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl]-4-phenyl-1-azoniabicyclo[2.2.2]octane chloride (SR 140333) induced a decrease in the slow wave frequency and duration of the activity periods evoked by distention, which makes it likely that NK1 receptor activation plays a role in the normal physiological distention-induced generation of peristaltic motor patterns. In summary, NK1 receptors play a role in normal development of peristalsis and NK1 receptor activation markedly increases propulsive peristaltic contractile activity.

Peristaltic motor activity of the gastrointestinal tract consists of co-ordinated movements of the gastrointestinal musculature resulting in mixing of content and allows for the aboral propulsion of contents (Costa and Furness, 1982). The control of various peristaltic motor patterns may involve the central nervous system, the enteric nervous system, and the muscle layers. In addition, recent evidence is accumulating for a prominent role of interstitial cells of Cajal (Thuneberg, 1982; Sanders, 1996; Huizinga et al., 1997). The absence of ICC associated with Auerbach’s plexus (ICC-AP) results in absence of normal distention-induced peristaltic activity (Der-Silaphet et al., 1998). ICC-AP generate the electrical slow waves (Koh et al., 1998; Thomsen et al., 1998), which determine the frequency and propagation characteristics of the intestinal propulsive contractions (Huizinga et al., 1998). In a model of distention-induced peristalsis, coordination of slow waves and enteric neural activity was seen to result in propagating contractile activity, leading to evacuation of content (Huizinga et al., 1998). Periods of propagating contractile activity alternated with mechanically quiescent periods (Donnelly et al., 2001). The duration of the activity or bursting periods and the duration of the quiescent periods describe the excitation of the tissue. Longer activity periods imply a faster aboral propagation of the intestinal contents because of the higher number of propagating contractions occurring in a certain time period (Waterman et al., 1994; Huizinga et al., 1998). In addition, an increase in slow wave frequency results in an increase in the number of propagating contraction waves per unit time, hence also increasing peristalsis. The first objective of the present study was to analyze distention-induced electrical activity quantitatively.
to facilitate analysis of pharmacological alterations in these motor patterns.

Distention directly activates enteric neurons (Brookes et al., 1999; Spencer et al., 2002; Vanner and MacNaughton, 2004). Distention can induce rhythmic activation of excitatory and inhibitory neural activity in segments of the colon (Spencer et al., 2002) or small intestine (Donnelly et al., 2001) such that periods of peristaltic activity alternate with periods of relative mechanical quiescence. Cholinergic activity is a prominent component of peristalsis, but in the absence of cholinergic activity, normal peristalsis can occur, indicating critical roles for other excitatory neurotransmitters. Although substance P is an excitatory neurotransmitter in the gut (Bartho and Holzer, 1985), its role in peristalsis is controversial. Holzer and colleagues concluded that substance P was inhibitory based on the observed increase in threshold for peristalsis: a higher level of distention was needed in the presence of NK1 agonists to elicit peristalsis (Holzer et al., 1995; Shahbazian and Holzer, 2000). Hence, the second objective of the present study was to evaluate the role of NK1-mediated substance P action in distention-induced peristalsis using additional parameters to evaluate peristaltic activity.

The slow waves, initiated by ICC, propagate into the musculature (Liu and Huizinga, 1993; Publicover, 1995) where action potentials can be generated upon neural excitation occurring superimposed on the slow waves, resulting in propagating contractile activity. It is not known whether substance P can affect the slow wave activity in the mouse intestine, although substance P mRNA is found in pacemaker ICC (ICC-AP) in the mouse (Epperson et al., 2000) and NK1 receptors are found on ICC-AP in the rat and guinea pig (Portbury et al., 1996; Vannucchi et al., 1997). Our third objective was to find evidence for a functional role of the NK1 receptors on ICC.

Materials and Methods

Female mice (Charles River Canada, Montreal, PQ, Canada), 3 to 4 months old, were sacrificed by cervical dislocation. The small intestine was exposed by a midline abdominal incision. The intestine was placed in a continuously oxygenated (95% O₂, 5% CO₂) Krebs' solution, pH 7.3 to 7.4. A 6-cm segment was taken 2 cm distal of the pyloric sphincter. The segment was placed in a 60-ml organ bath filled with continuously oxygenated (95% O₂, 5% CO₂) Krebs' solution at 37°C (Huizinga et al., 1998) The oral part was attached to a pressure column, a large Krebs' filled beaker that allowed for the maintenance of constant pressure. The distal part was attached to an open vertical tube that did not allow outflow of contents. The lumen of the segment was filled with Krebs' solution. The pressure within the segment was increased by increasing the level in the Krebs'-filled beaker. A level of 2.5 cm of fluid caused a sufficient distention to evoke peristalsis. The peristalsis could be visualized by propagating waves of circular smooth muscle contraction, causing partial occlusions and fluctuations in the height of the Krebs' solution in the vertical tube at the distal end of the segment. The intraluminal pressure was measured at two points in the segment with two Krebs' solution-filled plastic open-ended tubes whose openings were located 3 and 5 cm from the oral end of the segment. The electrical activity was measured by three suction electrodes attached to the serosal side of the segment, placed at regular 2-cm intervals starting 1 cm after the proximal end. The ground and recording electrodes were silver chloride-coated silver wires 0.06 mm in diameter. The recording electrodes were insulated by placing them in an open-ended flexible plastic tubing of 0.2 mm inner diameter and 1.0 mm outer diameter. The suction on the recording electrodes was 380 to 395 mm Hg.

Female guinea pigs (Hartley) weighing 100 to 150 g were sacrificed by inhalation of CO₂ gas in an enclosed container. Intestinal segments were obtained and handled in the same manner as the intestinal segments of the mouse. Drugs were added to the serosal side of the intestinal segments. All signals were amplified and recorded by Grass ink writing amplifier-recorder (7 PCM 12 C). All recordings were made within 2 h of removing the tissue from the animal.

Solutions and Drugs. The composition of the Krebs' solution was 120.3 mM NaCl, 5.9 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgCl₂, 20.0 mM NaHCO₃, 1.2 mM NaH₂PO₄, and 11.5 mM glucose. The solution was continuously oxygenated (95% O₂, 5% CO₂) at a pH of 7.3 to 7.4 and was kept at 37°C. SR 140333 was generously provided by Sanofi Synthelabo (Montpellier, France), dissolved in dimethyl sulfoxide, and diluted with nanopure water to a stock concentration of 10⁻³ M. SPF [Sar⁹,Met(O²)¹¹] obtained from Sigma-Aldrich (St. Louis, MO) was dissolved in nanopure water (10⁻³ M). GR 73632, obtained from Multiple Peptide Systems (San Diego, CA), was dissolved in nanopure water and diluted to 10⁻⁴ M.

Data Processing and Statistical Analysis. The duration of the quiescent and activity periods, the frequency of the slow waves in the different periods, the total average frequency, the amplitude of the slow waves, and the number of action potentials superimposed on the slow waves per unit time were determined. Active periods are characterized by slow waves with superimposed action potentials. These action potentials are easily identified when the experiment is in progress, and the generation of action potentials can actually be observed with fast twitches of the pen recorder. Observation of many recordings of the actual generation of action potentials makes it easy to recognize them once registered. Without observing the actual experiments, they are most easily recognized when they have a relatively high amplitude. Quiescent periods have slow wave activity without action potentials. Five-minute periods in the mouse experiments and 10-min periods in the guinea pig experiments were analyzed before and after the drug was added to the organ bath. All data were presented as mean ± S.E.M. To determine drug effects, the standard Student's t tests or the Wilcoxon rank test, when applicable, were used.
Results

Mouse. The present study gives a detailed and quantitative analysis of distention-induced motor patterns (Figs. 1–3) that were described qualitatively by Huizinga et al. (1998). Upon distention, resulting in intraluminal pressures of 2 to 4 cm of H2O, periods of activity developed (slow waves with superimposed action potentials associated with phasic contractions) alternating with relatively quiescent periods (where no superimposed action potentials and consequentially no phasic contractions were observed) \( n = 33 \). Furthermore, the slow wave frequency and amplitude were higher in the activity periods compared with the quiescent periods (Figs. 1 and 3). In the presence of distention, causing the described increase in intraluminal pressure, the duration of the quiet and activity periods were, respectively, \( 103.8 \pm 13.9 \) and \( 167.8 \pm 13.7 \) s \( P < 0.05; n = 26 \); the slow wave frequency, \( 37.7 \pm 1.0 \) and 39.6 \pm 1.0 cycles/min \( P < 0.00005 \); and the average amplitude, \( 1.7 \pm 0.2 \) and 2.7 \pm 0.2 mV \( P < 0.00001 \). The number and amplitude of the action potentials superimposed on a slow wave were related to the amplitude of the accompanying intraluminal pressure wave (Fig. 2), which was followed by an increase in the fluid level in the distal tube, implying a movement of luminal contents in the aboral direction. The jejunal \( n = 17 \) and ileal \( n = 9 \) segments of the small intestine showed a significant difference in frequency in both quiescent and activity periods. The slow wave frequency in the quiescent period was 40.3 \pm 1.3 and 35.4 \pm 1.3 cycles/min \( P < 0.05 \) in the jejunal and ileal segments, respectively. During the activity periods, the frequencies increased to 43.1 \pm 1.1 \( P < 0.01 \) and 37.3 \pm 1.2 \( P < 0.01 \) cycles/min, respectively.

To evaluate whether endogenous substance P acting through NK1 receptors was involved in generation of the activity periods, the concentration-dependent effects of the nonpeptide-selective NK1 receptor antagonist SR 140333 (Emonds-Alt et al., 1993; Croci et al., 1995) were studied (Figs. 3 and 4). SR 140333 \( 10^{-8} \text{M}; n = 7 \) caused a decrease in the duration of the activity periods from 235.3 \pm 23.9 to 121.5 \pm 43.9 s. It caused a decrease in the slow wave amplitude in the activity periods from \( 1.51 \pm 0.24 \) to 0.56 \pm 0.12 mV and a decrease in the number of slow waves carrying action potentials from 34.3 \pm 2.5 to 22.0 \pm 2.0 \( P < 0.01 \) per minute. This inhibition indicates that substance P-containing intrinsic nerves activated by distention and substance P acting through NK1 receptors contributed to the generation of distention-induced motor activity. The decrease in slow wave frequency in both activity and quiet periods by SR 140333 (reduction in total frequency from 11.5 \pm 1.1 to 6.9 \pm 0.8 cpm; \( P < 0.01 \)) suggests a NK1 receptor-mediated effect on ICC (see Discussion) (Fig. 4).

The NK1 receptor agonist SPF showed an excitatory effect by increasing the duration of the activity periods (Figs. 3 and 5–7). SPF \( 10^{-7} \text{M}; n = 8 \) increased the duration of the active periods from 118.6 \pm 22.6 to 199.2 \pm 18.8 s \( P < 0.01 \), induced action potentials superimposed on slow waves (number of slow waves with superimposed action potentials increased from 22.5 \pm 2.0 to 31.3 \pm 2.7/min; \( P < 0.001 \)), decreased the duration of the quiescent periods from 56.7 \pm 21.5 to 32.0 \pm 20.4 s \( P < 0.05 \), and increased slow wave frequency during activity periods from 40.2 \pm 3.1 to 41.2 \pm 3.2 cycles/min.

Fig. 2. Action potentials superimposed on slow waves were accompanied by transient increases in intraluminal pressure.

Fig. 3. Effects of SPF, GR 73632, and SR 140333 on distention-induced electrical and motor activity. Action potential generation, duration of activity and quiet periods, and slow wave amplitude and frequency were expressed relative to control \( = 1 \). The effects on the slow wave frequency suggest an NK1-mediated effect on the ICC. Top left, excitatory effects of SPF. Top right, effects of GR 73632. Bottom left, inhibitory effects of SR 140333. Bottom right, duration of the periods of quiescence were markedly increased by SR 140333.

Fig. 4. The effect of SR 140333, a selective NK1 antagonist on distention-induced activity. Upon distention (3 cm of H2O), a slow wave pattern was observed with activity and quiet periods (no superimposed action potentials visualized by dark peaks on top of the slow waves). The addition of SR 140333 caused a marked increase in the duration of the quiet periods. In this experiment, the duration of the quiet periods increased from 13.0 to 32.6 s, the slow wave frequency in the quiet periods decreased from 41.4 to 37.0 cycles/min, and slow wave amplitude decreased from 1 to 0.7 mV. In this particular experiment, the duration of the activity periods did not change and ranged from 7.3 to 8.1 s under both conditions. The slow wave frequency in the activity periods was 43.4 cycles/min before and 42.5 cycles/min after SR 140333, and the slow wave amplitude decreased from 1.5 to 1.6 mV. The occurrence of slow waves with superimposed action potentials was reduced from 14.9 to 8.5/min.
The selective NK1 receptor agonist GR 73632 had a strong excitatory effect on the parameters describing the peristalsis (n = 7) (Figs. 3 and 8). These effects were comparable with the effects of SPF. The excitatory effect of GR 73632 (10^{-7} M; n = 7) was reflected in an increase in the generation of action potentials, the number of slow waves carrying action potentials increased from 34.8 \pm 1.3 to 38.4 \pm 1.5 cpm (P < 0.01), the duration of the activity periods from 168.3 \pm 36.2 to 234.9 \pm 25.9 s (P < 0.05), and the slow wave frequency in the activity periods from 38.5 \pm 1.4 to 39.7 \pm 1.4 cpm (P < 0.01). Also significant was the change in slow wave amplitude during activity periods, which increased from 1.26 \pm 0.29 to 1.74 \pm 0.44 mV. The actions of GR 73632 in the presence of atropine were similar (n = 4).

When SR 140333 was given when GR 73632 was already present in the organ bath, the excitatory activity was strongly inhibited (n = 6). The generation of action potentials, the duration of the activity periods, and the total frequency were decreased. SR 140333 caused a decreased number of slow waves carrying action potentials from 26.4 \pm 3.1 to 16.6 \pm 4.5/min, a decrease in duration of activity periods from 189.7 \pm 23.6 to 102.2 \pm 28.4 s (P < 0.01), and a decrease in average frequency from 37.3 \pm 1.0 and 34.8 \pm 1.3 cycles/min (P < 0.01).

**Guinea Pig.** In the guinea pig, intraluminal pressure of 3.5 cm of H2O induced peristaltic contractions with associated electrical activity in the intestinal segment. However, without distention, no electrical or mechanical activity was observed in contrast to the intestinal segments of the mouse where slow waves are always observed and sometimes superimposed action potentials, occasionally in a bursting manner. Upon distention of the guinea pig intestinal segment, slow waves were evoked. Every slow wave was associated with a change in intraluminal pressure and change of fluid level in the distal tube, implying a movement of luminal contents in aboral direction (Fig. 9). The slow waves were also 1:1 related with indentations in the muscle wall of the segment. The slow waves, the indentations in the muscle wall, and the intraluminal pressure changes propagated aborally. The induced slow waves were almost always associated with action potentials. The pattern consisted either of continuous slow wave activity with superimposed action potentials or of alternating activity periods and quiet periods (in which no electrical activity, no changes in intraluminal pressure, and no contractions of the tissue were observed). With a distention at an intraluminal pressure of 3.5 cm, the slow wave frequency was 8.3 \pm 0.6 cycles/min (n = 8).

The effects of the NK1 receptor agonist SPF and the selec-

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**Fig. 5.** SPF (10^{-6} M) increased duration of activity periods, and the amplitude and frequency of slow wave activity. In this experiment, the duration of the quiet periods changes from 47.5 to 12.3 s. Within the quiet periods, the slow wave frequency changed from 42.6 to 39.7 cycles/min; the amplitude did not change. The duration of the activity periods changed from 33.9 to 39.4 s. Within the activity periods, the slow wave frequency changed from 45.7 to 47.1 cycles/min. In the presence of SPF, the slow wave amplitude in the quiet and activity periods was 2.3 and 2.6 mV, respectively. The occurrence of slow waves with superimposed action potentials changed from 17.2 to 23.8/min.

**Fig. 6.** SPF (10^{-8} M) increased intensity of action potential generation. In this experiment, the duration of activity periods increased from 24 to 30 s, the slow wave frequency increased from 42.5 to 42.9 cycles/min, and the average slow wave amplitude increased from 1.1 to 1.5 mV. Action potentials increased in amplitude and in the number of action potentials on each slow wave.

3.0 cpm (P < 0.05). The increase in frequency suggests an NK1 receptor-mediated effect on ICC.

When the NK1 receptor antagonist SR 140333 (10^{-8} M) was present in the organ bath, SPF did not show any effect on the generation of action potentials, frequency, amplitude, or duration of both activity and quiescent periods at the concentrations of 10^{-6} and 10^{-7} M (n = 5). When SR 140333 was given when SPF was already present in the organ bath (n = 5), SR 140333 inhibited both the effects of extrinsic and intrinsic substance P on generation of action potentials, and slow wave frequency and amplitude. The most marked effect was seen on the duration of quiet periods, which changed from 40.6 \pm 9.0 to 201.5 \pm 47.1 s (P < 0.05) because of exposure to SR 140333 (10^{-7} M). The duration of the activity periods changed from 140.3 \pm 29.5 to 33.4 \pm 18.4 s (P < 0.05). The selective NK1 receptor agonist GR 73632 had a strong excitatory effect on the parameters describing the peristalsis

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**Fig. 7.** SPF (10^{-6} M) increased the duration of the activity periods during a continuous distension of 3 cm of H2O. In this experiment, SPF increased the duration of activity periods from 29.7 to 43.1 s and the slow wave frequency from 37.3 to 40.9 cycles/min, and the average slow wave amplitude increased from 1.8 to 1.9 mV. The duration of the quiet periods changed from 15.6 s to 3 s. The number of slow waves carrying action potentials changed from 23.8 to 37.9/min. In this figure, note the large difference between slow waves with and without superimposed action potentials.
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Fig. 8. Excitatory effects of GR 73632 (10⁻⁸ M). GR 73632 has excitatory action on the intestine, similar to SPF. In this experiment, in response to GR 73632 (10⁻⁸ M), the average duration of the quiet periods changed from 13.9 to 7.8 s, the slow wave frequency during the quiet periods did not change, and the slow wave amplitude decreased from 2.7 to 2.5 mV. The duration of the activity periods changed from 23 to 50 s. Within the activity periods, the slow wave frequency increased from 31.3 to 33.1 cycles/min and the slow wave amplitude from 2.8 to 4.2 mV. The number of slow waves with superimposed action potentials increased from 19.3 to 26.8/min.

Fig. 9. The distension-induced slow wave pattern in the guinea pig and the effects of GR 73632 on the duration of the activity periods and the slow wave frequency. A, under control conditions, periods of electrical quiescence alternate with periods of slow wave activity with superimposed action potentials. In this experiment, the average duration of the activity periods was 173 s, and the average duration of the quiet periods was 427 s. The frequency of the slow waves was 9.2 cycles/min. Note that the change in intraluminal pressure is directly related to the occurrence of slow waves with superimposed action potentials (B). In the presence of GR 73632, quiet periods were not observed; the frequency of the slow waves increased to 16.0 cycles/min.

2.2 cycles/min after exposure (P < 0.05) (n = 3).

Discussion

The model of distention-induced peristalsis used in the present study allowed the investigation of motor patterns developing in response to physiological levels of general distention in a segment of the proximal small intestine of the mouse and guinea pig. Interestingly, the dominant motor activity presented as cyclic activity, characterized by periods of propagating slow waves with superimposed action potentials alternated with quiescent periods consisting of slow waves without action potentials in the mouse and electrical quiescence in the guinea pig. Slow waves with superimposed action potential were associated with propagating intraluminal pressure waves. The present study shows that the selective NK1 receptor agonists SPF and GR 73632 prolong the duration of the activity periods of distention-induced motor patterns. The NK1 antagonist SR 140333 induces a decrease in the slow wave frequency and duration of the activity periods evoked by distention, which makes it likely that NK1 receptor activation plays a role in the normal physiological distention-induced generation of peristaltic motor patterns. These observations indicate that the duration of the activity periods depends in part on actions of substance P mediated by the NK1 receptor. Longer activity periods imply a faster aboral propagation of the intestinal contents because of the higher number of propagating contraction occurring in a certain time period. In addition, an increase in slow wave frequency during the activity periods is excitatory since more slow waves with superimposed action potentials (hence intraluminal pressure waves) occur per unit time. Furthermore, increased intensity of generation of action potentials caused increase in force of contraction consistent with the observations of Holzer in the rabbit ileum (Holzer, 1982). A slow wave with superimposed action potentials occurs virtually simultaneously around the circumference of the intestine in the circular muscle layer and propagates aborally causing a local propagating contraction of the muscle that has a powerful mixing effect and moves the contents of the lumen (Diamant and Bortoff, 1969; Sarna and Otterson, 1989; Lammers et al., 1996; Huizinga et al., 1997).

Holzer and coworkers studied the effect of substance P on peristaltic activity in a different manner and recorded different parameters. An ileal segment of the guinea pig intestine was infused at a constant rate of 0.5 ml/min, and the pressure was allowed to build up until threshold of peristalsis was reached whereupon the segment was allowed to empty at which point the process started anew (Holzer et al., 1995). Substance P caused a prompt but transient (7- to 10-min) stimulation of peristalsis shown as increase in frequency and decrease in pressure threshold of the peristaltic waves. Thereafter, the frequency became slower and the threshold higher. The NK1 agonist substance P methyl ester failed to stimulate the peristaltic response but showed a delayed SPF and GR 73632 suggests an NK1 receptor-mediated effect on the ICC.

With SPF or GR 73632 present in the organ bath, SR 140333 inhibited the excitatory effects of SPF and GR 73632. The slow wave frequency decreased, suggesting an NK1 receptor-mediated effect on the ICC. The slow wave frequency was 7.6 ± 1.4 cycles/min before exposure to SR 140333 and 5.9 ± 2.2 cycles/min after exposure (P < 0.05) (n = 3).
inhibition, whereas the NK3 agonist succinyl-[Asp6,N-MePhe8]-substance P-(6-11) potently stimulated peristalsis (Holzer et al., 1995). Interestingly, although in Holzer’s experiments the pressure threshold increased upon NK1 stimulation (mediated by NO), the rate of rise of the intraluminal pressure upon fluid infusion also increased (see Figs. 1 in both Holzer (1997) and Shahbazian and Holzer (2000)), which caused the number of peristaltic waves per minute to increase. In our experiments, we did not allow the intestinal segment to empty, leaving the intraluminal pressure relatively constant. When peristaltic activity was induced in vivo by contrast fluid delivered by stomach emptying (Der-Silaphet et al., 1998), rapid emptying of the proximal duodenum was observed, but the jejunal segment remained filled for long periods with continuing peristaltic activity, identical to our experimental setup. Hence, peristaltic contractile activity may under such circumstances act primarily to mix content. The data from Holzer’s work and our study together suggest that despite the increase in pressure threshold, the overall effect of substance P, mediated by NK1 receptors, is an increase in distention-induced peristaltic activity.

SPF and GR 73632 caused an increase in slow wave frequency. SR 140333, the selective NK1 receptor antagonist, decreases the slow wave frequency. This indicates that part of the excitation of the musculature involves action on NK1 receptors on ICC. Slow waves of the small intestine originate in ICC-AP (or myenteric plexus) (Ward et al., 1994; Huizinga et al., 1995). The slow wave frequency is set by intracellular factors intrinsic to ICC (Suzuki et al., 2000; Ward et al., 2000; Malyss et al., 2001), and a change in slow wave frequency by NK1 receptor activation is therefore mediated by NK1 receptors on ICC-AP. Indeed, NK1 immunoreactivity was shown to be present on ICC-AP of the guinea pig (Portbury et al., 1996; Vannucchi et al., 1997) and rat (Sternini et al., 1995; Vannucchi et al., 1997). In the mouse ICC-AP contain mRNA for the NK1 receptor (Epperson et al., 2000). Pharmacological evidence suggests that the contractile effect observed after stimulation of the NK1 receptor is because of a direct excitation of smooth muscle cells (Bartho and Holzer, 1985; Maggi et al., 1994a,b; Holzer and Holzer-Petsche, 1997). The muscle layers of the gut receive a dense supply of tachykinin-containing nerve fibers, most of which originate from intrinsic enteric neurons. Tachykinin containing neurons connect the ganglia within the myenteric and submucosal plexus and issue projections to the longitudinal muscle and circular muscle (Bartho and Holzer, 1985; Holzer and Holzer-Petsche, 1997). Distention-induced changes in motility may be mediated by ICC associated with the deep muscular plexus (Lino et al., 2004).

NK1 receptor activation showed an increase in slow wave amplitude as monitored with extracellular electrodes. Flexible extracellular electrodes are used because they can easily adapt to changes in electrode position because of distention. However, such electrodes do not measure the absolute value of the slow wave amplitude, and changes in amplitude can be caused by a variety of factors. The electrode is positioned within the musculature and records a compound slow wave from extracellular signals to which many neighboring smooth muscle cells contribute. In fact, the more extensive the smooth muscle cells are electrically coupled, the more smooth muscle cells will contribute to the signal. Since intracellular recording of slow waves does not reveal a marked increase in slow wave amplitude upon excitation (El-Sharkawy and Szurszewski, 1978; Huizinga et al., 1984), the most likely cause of the increased slow wave amplitude is an increase in intercellular coupling in the musculature by NK1 activation. Electrical coupling is not extensively studied as a mechanism of tissue excitability, but it is subject to modulation; for example, glucose-induced increased in electrical coupling in islet cells is a factor in glucose-induced insulin secretion (Meda et al., 1984).

In summary, the extent to which distention induces peristaltic activity is in part regulated via NK1 receptor activation. In addition, pharmacological activation of the NK1 receptor markedly increases the duration and intensity of peristaltic activity that is induced by distention. Distention induces periodic appearance of five to 10 or more peristaltic waves of aborally propagating contractions. NK1 receptor activation prolongs these activity periods, and within these periods increases slow wave frequency, slow wave amplitude, and extent of generation of action potentials. Although NK1 receptor activation increases the threshold volume of distention for induction of peristalsis (Shahbazian and Holzer, 2000), it also promotes the rate of rise of intraluminal pressure (Holzer, 1997) such that, taking into account the results of the present study, the overall effect on peristalsis is markedly excitatory.

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