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# The Nature of Noncholinergic Membrane Potential Responses to Transmural Stimulation in Guinea Pig Ileum

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**The effect of substance P antagonism on membrane potential responses to transmural nerve stimulation in the presence of atropine was examined in circular smooth muscle of the guinea pig ileum. Intracellular recordings of membrane potential responses recorded 3–5 mm oral to the transmural stimulus consisted of an inhibitory junction potential followed by two distinct depolarizations referred to as early and late excitatory junction potentials. Substance P antagonism was achieved by desensitization with high doses of substance P or use of the antagonist Spantide (Sigma Chemical Co., St. Louis, MO). Substance P antagonism had no effect on the amplitude of the inhibitory junction potential, caused an increase in the latter portion of the early excitatory junction potential, and abolished the late excitatory junction potential. The excitatory junction potential potentiated by substance P receptor antagonism was associated with a decrease in membrane resistance, increased in amplitude with conditioning hyperpolarizations to the estimated equilibrium potential for $K^+$, and was blocked by the $Cl^-/HCO_3^-$ exchange inhibitor DIDS or prolonged perfusion with low-chloride solution. These studies suggest that a noncholinergic, non–substance P neurotransmitter is released from enteric motoneurons that produces excitation through an increase in smooth muscle chloride conductance.**

The propulsion of material within the small intestine involves a peristaltic reflex that was originally described by Starling as the "law of the intestine." This reflex consists of a contraction oral to a distending stimulus and a relaxation followed by a contraction anal to the stimulus (1). Previous studies have attempted to examine the pathways of intrinsic noncholinergic inhibitory and excitatory nerves involved in this reflex by characterizing the membrane potential responses of circular muscle at various sites oral and anal to a transmural nerve stimulus in the presence of atropine (2–4). Recent studies by us have shown that the membrane potential responses at sites immediately oral to a short train stimulus consist of an inhibitory junction potential (IJP) followed by two distinct components of depolarization referred to as early and late excitatory junction potentials (early EJP and late EJP) (4).

A number of studies have suggested that substance P–containing nerves play an important, if not exclusive, role in the noncholinergic excitatory responses of the peristaltic reflex (5–8). However, several studies have suggested that an excitatory response is observed following nerve stimulation in the presence of atropine and substance P antagonists (9,10). The purpose of the present investigation was to examine the ionic mechanisms responsible for this excitatory response.

Our studies demonstrate that an excitatory junction potential in response to a short train of transmural nerve stimulation is observed in the presence of muscarinic and substance P antagonism and that the ionic basis for this excitatory event is an increase in membrane chloride conductance.

## Materials and Methods

Twenty-two (male or female) guinea pigs weighing between 250 and 400 g were anesthetized by means of $CO_2$ narcosis and subsequently stunned and bled through the carotid arteries. A segment of ileum 15–20 cm in length was

*Abbreviations used in this paper:* DIDS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid; DMSO, dimethyl sulfoxide; EJP, excitatory junction potential; IJP, inhibitory junction potential; RMP, resting membrane potential.


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taken from between 5 and 40 cm oral to the ileocecal junction and the anal aspect was marked with a black silk suture. The segment was then cleansed with an intraluminal flush of Krebs solution and cut open along the mesenteric border of its longitudinal axis. The segment was then trimmed to a length of 100 mm and transferred to a bath where it was pinned with the mucosal surface facing down. The bath consisted of a chamber 130 mm in length and 20 mm in width with a floor of Sylgard (Dow-Corning, Midland, MI). The bath had a volume of 14 mL and was continuously perfused with Krebs solution which was oxygenated and heated before entry into the bath. The flow was maintained at a constant rate of 3 mL/min. The bath fluid was further oxygenated by bubbling of 95% $O_2$-5% $CO_2$ directly into the bath. The bath temperature was maintained at 29.5 ± 1.0°C rather than 37°C so as to decrease muscle contractions and displacement of the recording microelectrode. The Krebs solution consisted of the following (in mmol/L): glucose, 11.5; bicarbonate, 21.9; phosphate, 1.2; sodium, 138.5; calcium, 2.5; magnesium, 1.2; potassium, 4.6; and chloride, 125. The pH of the Krebs solution after 30 minutes of bubbling with 95% $O_2$-5% $CO_2$ ranged between 7.36 and 7.45. Atropine (1 μmol/L) and nifedipine (0.1 μmol/L) were added to the perfusate to block muscarinic transmission and reduce spontaneous and evoked contraction of the muscle. Organic calcium entry blockers such as nifedipine (0.1 μmol/L) have been shown to lack any significant effects on synaptic transmission in enteric nerves (2,11–13).

### *Intracellular Recording*

Intracellular recordings of membrane potential were obtained from smooth muscle cells using microelectrodes made from glass of 1.2 mm external diameter (Frederick Haer & Co., Brunswick, ME) and filled with 1 mol/L potassium methyl sulfate. The resistance of the microelectrodes was between 30 and 80 MΩ. The microelectrode was connected to the probe of a high-input impedance electrometer (Neuroprobe 1600, A-M Systems Inc., Everett, WA), the output of which was displayed on a digitizing storage oscilloscope (Tektronix 5223, Tektronix Inc., Beaverton, OR). Permanent records were made by passing the oscilloscope signals onto a strip chart recorder (Gould 220, Gould Inc., Cleveland, OH).

Impalement of a circular smooth muscle cell was made by advancing the microelectrode attached to a micromanipulator (E. Leitz Inc., Rockleigh, NJ) through the surface longitudinal muscle and then into the deeper cell layer of circular muscle. Previous studies have demonstrated that, in response to a single pulse of transmural stimulation, IJPs are observed only in circular muscle and not longitudinal muscle impalements (14). Hence, a successful impalement of a circular smooth muscle cell was defined as a negative deflection in the oscilloscope trace with subsequent maintenance of a stable negative potential for longer than 10 minutes and an IJP in response to a single pulse of transmural stimulation. All membrane potential values were determined by the difference between the stable potential recorded within the cell compared with the balanced zero potential upon withdrawal. Transmural stimulation of intramural nerves within the intestinal strip was performed by means of two Ag-AgCl electrodes (0.01 inch diameter) placed above and below the intestinal preparation and perpendicular to its longitudinal axis. The stimulating electrodes were positioned 3–5 mm anal to the recording microelectrode and connected to a stimulator (Grass S-88, Grass Instruments, Quincy, MA) in series with a stimulus isolation unit (Grass SIU5) and a constant current unit (Grass CCU1). The transmural nerve stimulus consisted of a 200-millisecond train of pulses (2.0 milliseconds pulse duration, 15 mA, 20 Hz). All membrane potential responses to this train of transmural stimulation were abolished by tetrodotoxin (1 μmol/L) thereby insuring that they were neurogenic in origin.

### *Conditioning Hyperpolarizations*

In studies examining the effects of conditioning hyperpolarization on the nerve-mediated membrane potential events, large electrotonic potentials were frequently administered using the technique originally described by Abe and Tomita (15). The current that passed between the two stimulating plates in this bath was monitored by a constant current monitor unit (Grass) positioned in series between the plates and the stimulator. In these studies, the recording microelectrode was positioned within 2 mm from the stimulating plate adjacent to the recording compartment of the bath. Two Ag-AgCl electrodes (0.01 inch diameter) positioned above and below the intestinal preparation perpendicular to its longitudinal axis and approximately 3–5 mm anal to the recording microelectrode were used to deliver transmural nerve stimulation. Current leakage into the recording compartment during electrotonic stimulation produced small voltage differences (less than 6 mV) in the extracellular fluid between the microelectrode and the bath reference electrode. To minimize effects produced by this current leakage, after voltage recordings had been made in one cell, the microelectrode was dislodged from the cell and the same stimuli were delivered and extracellular voltages were recorded. These extracellular voltages were subtracted from the intracellular value to obtain the true electrotonic potential.

I-V curves were determined by passing hyperpolarizing or depolarizing currents 700 milliseconds in duration and of varying intensity as monitored by the constant current unit monitor. I-V curves were determined only in those preparations in which the largest extracellular voltages recorded were 3 mV or less.

### *Statistics*

Statistical comparisons were made using standard Student paired and unpaired *t* tests and covariance analysis. All data are expressed as mean ± SEM.

### *Drugs*

Drugs used in this study included atropine, tetrodotoxin, dimethyl sulfoxide (DMSO), 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (DIDS), substance P, Span-

tide, and nifedipine and were obtained from Sigma Chemical Co. (St. Louis, MO). Nifedipine was dissolved in 95% ethanol at 10 mmol/L and stored in a light-free container. The DIDS was dissolved in 10% DMSO at 60 mmol/L. Low-chloride solution was made by substituting sodium chloride with sodium isethionate (Sigma), resulting in a solution having a chloride concentration of 12.4 mmol/L.

## Results

### *Substance P Antagonism*

Substance P antagonism was achieved by (a) prolonged exposure to high concentrations of substance P (desensitization) and (b) the use of the known substance P antagonist Spantide.

### *Substance P Desensitization*

Perfusion with substance P (1 μmol/L) resulted in a rapid membrane depolarization associated with displacement of the electrode from the impaled smooth muscle cell. Attempts at long-term impalement during the first 5–10 minutes of substance P perfusion were invariably unsuccessful. However, following 30 minutes or more of perfusion, long-term impalements were easily attained and demonstrated resting membrane potentials slightly less than in controls (controls = −52.8 ± 1.6 mV; 30 minutes substance P (1 μmol/L) = −47.7 ± 1.7 mV; $P < 0.05$, n = 50 in five animals). Desensitization of substance P receptors was verified after 30 minutes of perfusion by the absence of any depolarizing effect on resting membrane potential upon increasing the perfusion concentration of substance P tenfold to 10 μmol/L.

I-V relationships before and after 30 minutes of exposure to substance P (1 μmol/L) did not demonstrate any significant changes in slope ($P > 0.05$), suggesting that substance P desensitization was not associated with any changes in membrane resistance (Figure 1*A*).

### *Substance P Antagonist*

Perfusion with Spantide (10 μmol/L) did not cause membrane depolarization or displacement of the recording electrode. Following 10 minutes of perfusion no significant change was observed in resting membrane potential (RMP) (controls = −51.3 ± 1.8 mV; Spantide = −50.5 ± 2.1 mV; $P > 0.05$, n = 30 in three animals).

I-V relationships before and after 10 minutes of exposure to Spantide (1 μmol/L) did not show any significant changes in slope ($P > 0.05$), suggesting that Spantide perfusion was not associated with any changes in membrane resistance (Figure 1*B*).

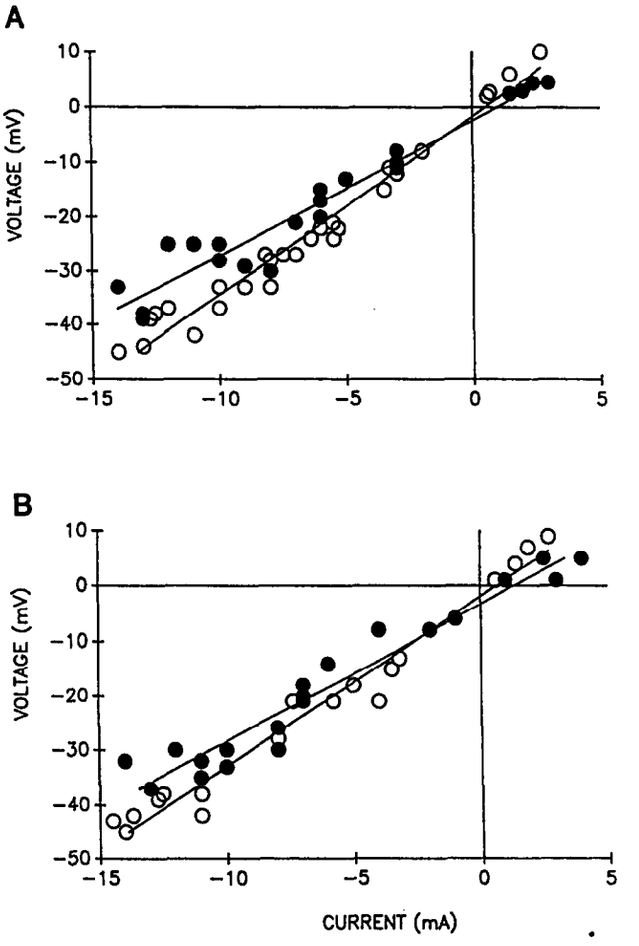


**Figure 1. I-V relationships in guinea pig ileum circular muscle before and after substance P antagonism. The results are plotted in six cells from three animals before (○) and after (●) substance P antagonism. The lines were fitted by first-order regression analysis and had *R* values > 0.97.**

*A.* Following 30 minutes of perfusion with substance P (1 μmol/L), a slight decrease was observed in the slope of the I-V curve which was not significantly different from that of controls in the same three preparations ($P > 0.05$).

*B.* Following 10 minutes of perfusion with the substance P antagonist Spantide, a slight decrease was also observed in the slope of the I-V curve which was not significantly different from that of controls ($P > 0.05$).

### *Effect of Substance P Antagonism on Nerve-Mediated Responses*

The membrane potential responses to nerve stimulation and the effect of substance P antagonism on these responses are shown in Figure 2*A*. The control responses consisted of an initial IJP followed by two distinct depolarizing events or EJPs. The first of these EJPs [previously referred to as the early EJP (4)] reached a maximal amplitude 2.9 ± 0.3 seconds from the stimulus (n = 16 in four animals) and per-

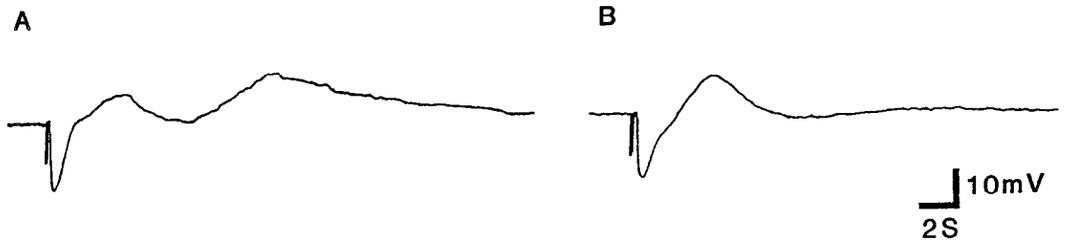


**Figure 2. Membrane potential responses to transmural nerve stimulation and the effect of substance P antagonism on these responses in a single preparation.**

*A.* Membrane potential responses consisted of an initial IJP followed by two distinct EJPs referred to as early EJP and late EJPs.

*B.* Substance P antagonism had no effect on the amplitude of the initial IJP but prolonged the duration of the IJP. This prolongation of the duration of the initial IJP appeared to be due to a decrease in amplitude of the initial portion of the early EJP. Substance P antagonism increased the amplitude of the latter portion of the early EJP and abolished the late EJP.

sisted until approximately 7 seconds after the stimulus. The subsequent EJP [previously referred to as the late EJP (4)] frequently began before the membrane potential had returned to its baseline resting value from the early EJP and reached a maximal amplitude 8.3 ± 0.4 seconds following the stimulus (n = 16 in four animals). The late EJP had a duration of 14.4 ± 2.4 seconds returning to baseline RMP 18.5 ± 2.1 seconds after the stimulus.

Figure 2*B* shows the membrane potential responses following substance P antagonism by means of substance P desensitization. Studies with Spantide showed similar effects. Substance P antagonism had no effect on the amplitude of the IJP but significantly prolonged its duration. This prolongation of the IJP appeared to be due to abolition of the initial portion of the early EJP. Substance P antagonism resulted in an increase in amplitude of the early EJP. The late EJP was abolished by substance P antagonism.

Quantitative data concerning the effects of substance P antagonism on the amplitude and duration of the IJP and the amplitudes of the early and late EJPs are provided in Figure 3.

The above studies were performed in the presence of nifedipine (0.1 μmol/L). To insure that this calcium channel blocker was not significantly altering the membrane potential responses observed both before and after substance P antagonism, we performed

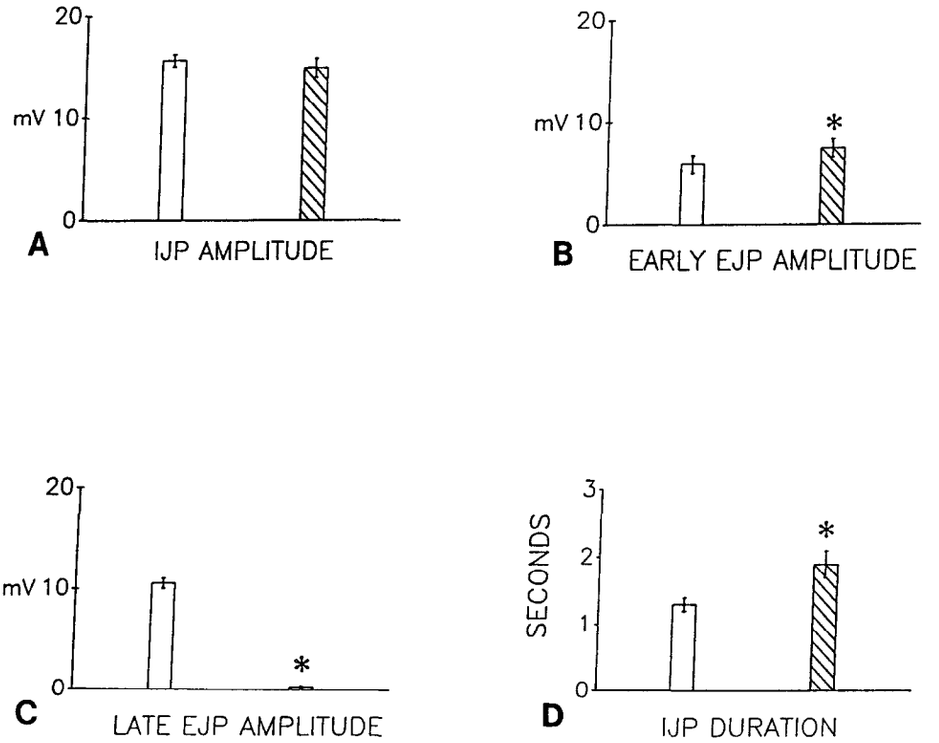


**Figure 3. Quantitative data concerning the membrane potential responses to transmural nerve stimulation and the effects of substance P antagonism on these responses.**

*A.* The amplitude of the IJP was not effected by substance P antagonism ($P > 0.05$, n = 16 in four animals).

*B.* The amplitude of the early EJP was significantly increased following substance P antagonism ($P < 0.05$, n = 16 in four animals).

*C.* The amplitude of the late EJP was markedly decreased following substance P antagonism ($P < 0.05$, n = 16 in four animals).

*D.* The duration of the IJP was significantly increased following substance P antagonism ($P < 0.05$, n = 16 in four animals).

recordings in the absence of nifedipine. As shown in Figure 4, transmural stimulation resulted in the previously described responses consisting of an IJP followed by two EJPs. However, in the absence of nifedipine, the late EJP had superimposed spike potentials which frequently resulted in displacement of the recording electrode from the impaled cell. Following substance P antagonism, the IJP was prolonged in duration (because of antagonism of the initial portion of the early EJP) and followed by the early EJP, which was associated with spike potentials and, frequently, displacement of the recording electrode. These studies verify previous studies suggesting that nifedipine has little effect on the membrane potential responses in the guinea pig ileum other than the blockade of spike potentials and associated muscle contraction (11,12).

*Effect of Conditioning Hyperpolarizations On Nerve-Mediated Responses*

Next, we examined the ionic mechanisms responsible for the IJP and early and late EJPs. Figure 5 shows the effect of conditioning hyperpolarizations on the membrane potential responses. Progressively greater conditioning hyperpolarizations resulted in a progressive (a) decrease in amplitude of the initial IJP, (b) decrease in amplitude of the initial component of the early EJP, (c) increase in amplitude of a rapid transient depolarization superimposed on the latter portion of the early EJP, and (d) decrease in amplitude of the late EJP. The progressive decrease in IJP amplitude with conditioning hyperpolarization and abolition at the estimated equilibrium potential for $K^+$ ($E_K$) (−85 mV) confirms previous studies suggesting that this IJP is due to an increase in membrane potassium conductance (16–18). Similarly, the decrease in amplitude of the initial portion of the early EJP and all of the late EJP with conditioning hyperpolarizations approaching the estimated $E_K$ suggests that these excitatory components might be due to a decrease in membrane potassium conductance.

The effects of conditioning hyperpolarizations on membrane potential responses following substance P antagonism are shown in Figure 6. Progressively greater conditioning hyperpolarizations following substance P antagonism were associated with a progressive decrease in amplitude of the initial IJP and increase in amplitude of a rapid transient depolarization superimposed on the EJP. The reversal potential for this EJP could be extrapolated from a plot of the relationship between hyperpolarizing conditioning potentials and amplitude of the EJP. As shown in Figure 7, the extrapolated zero value for the amplitude of the EJP was approximately 25 mV positive to RMP. Because RMP averaged −52 mV in this sample of 20 cells, the reversal potential extrapolated from this data would therefore be at a membrane potential of approximately −27 mV. The progressive increase in amplitude of the rapid transient depolarization with greater conditioning hyperpolarizations and extrapolated reversal at or near the known equilibrium potential for chloride raised the possibility that this

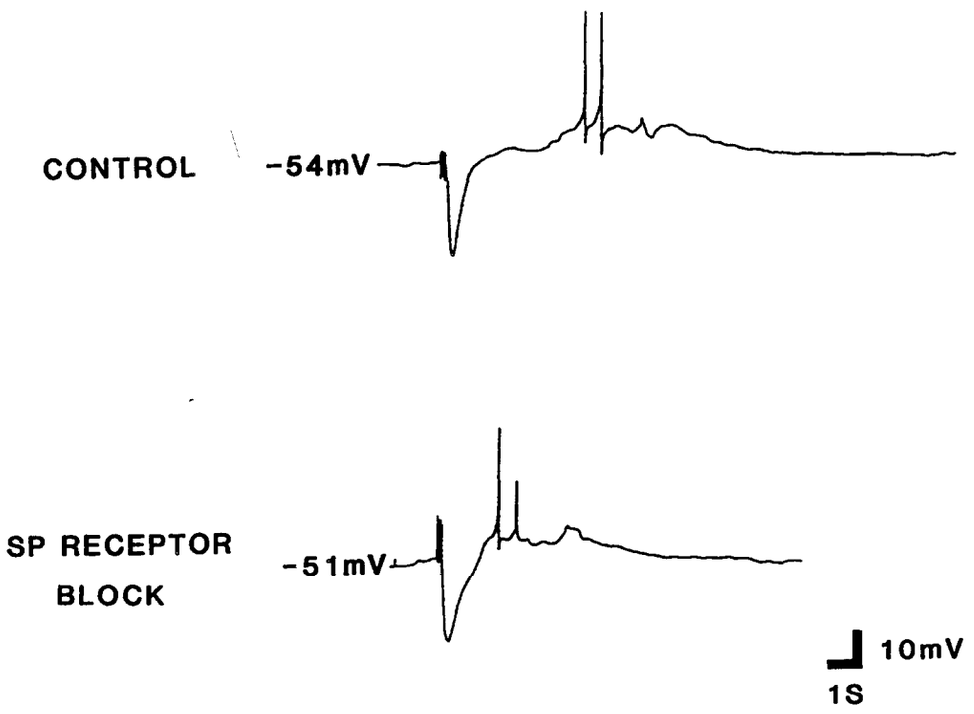


**Figure 4. Membrane potential responses to transmural nerve stimulation in the presence of atropine but not nifedipine. The control response consisted of an initial IJP followed by an early EJP and a late EJP. The late EJP was superimposed with spike potentials whose amplitudes were truncated by the channel width of the chart recorder. Following substance P antagonism, the IJP was unchanged and the late EJP was abolished. The initial portion of the early EJP was antagonized and the latter portion of the early EJP was superimposed with spike potentials.**

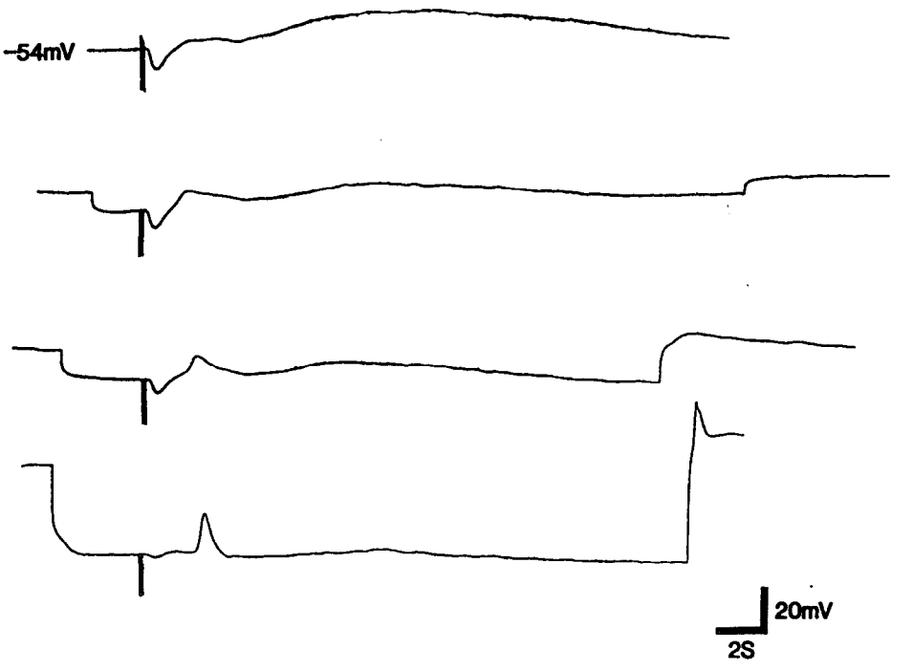


**Figure 5. Effect of conditioning hyperpolarizations on membrane potential responses to transmural nerve stimulation. The control response (RMP = −54 mV) consisted of an IJP followed by an early and late EJP. Progressively greater conditioning hyperpolarizations resulted in a progressive decrease in IJP amplitude, a progressive decrease in the initial portion of the early EJP, a progressive increase in the latter portion of the early EJP, and a progressive decrease in the late EJP. At −87 mV, the IJP and late EJP were almost abolished and a large rapid transient depolarization was observed that coincided temporally with the latter portion of the early EJP.**

event may be due to an increase in membrane chloride conductance.

*Membrane Conductance During the Excitatory Junction Potential Observed Following Substance P Antagonism*

These studies compared the amplitudes of electrotonic potentials resulting from 700-millisecond pulses of hyperpolarizing currents of constant intensity delivered at RMP and during the EJP produced by transmural nerve stimulation following substance P antagonism. As shown in Figure 8, a marked decrease was observed in amplitude of the electrotonic potential administered during the EJP as compared with that delivered at RMP. Studies in 12 cells in three different animals demonstrated a 52.8% ± 2.3% reduction in amplitude of the electro-

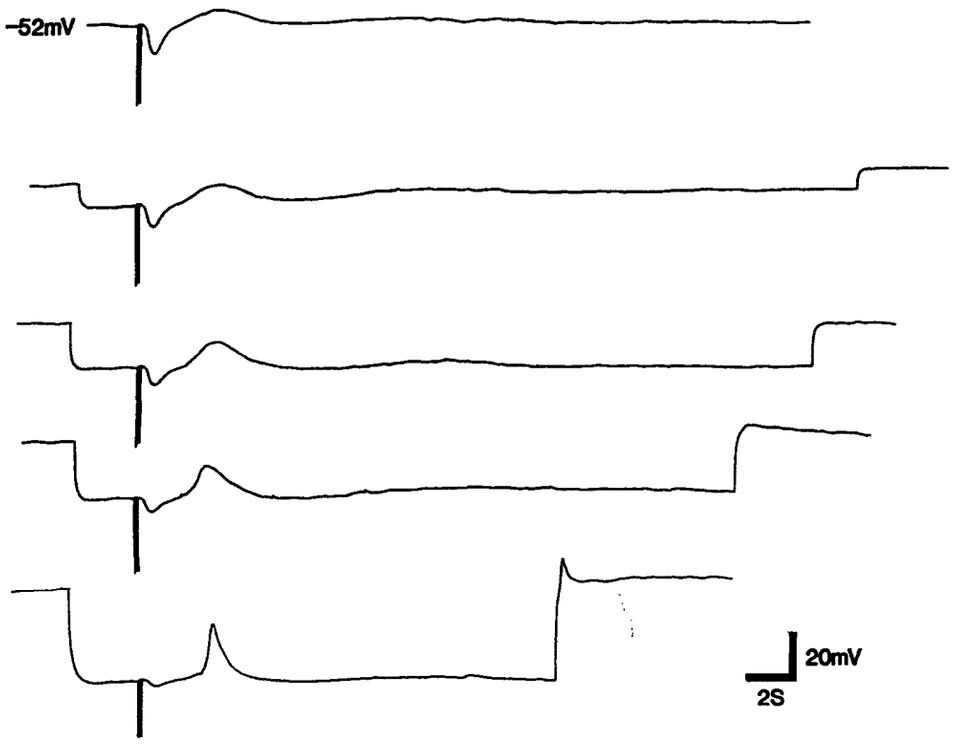


**Figure 6. Effect of conditioning hyperpolarizations on membrane potential responses following substance P antagonism. The control response (RMP = −52 mV) consisted of an IJP followed by an EJP. Progressively greater conditioning hyperpolarizations resulted in a progressive decrease in amplitude of the IJP and a progressive increase in amplitude of the EJP. At −85 mV, the IJP was almost abolished and there was a large rapid transient depolarization coinciding temporally with the EJP.**

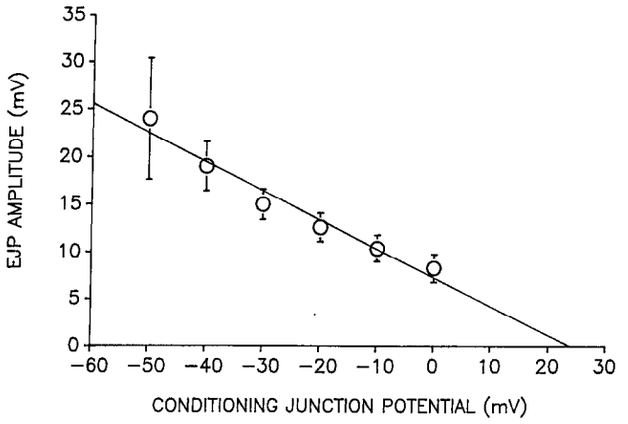


**Figure 7. Quantitative data on the effect of conditioning hyperpolarizations on the amplitude of the EJP present following substance P antagonism. The mean RMP in 20 cells in four animals was −52.4 ± 1.4 mV. Progressively greater conditioning hyperpolarizations up to 50 mV resulted in a progressive increase in amplitude of the EJP or rapid transient depolarization. The data were line-fitted by first-order regression analysis having an *R* value of 0.98. Extrapolation of this fitted line to the zero point of the EJP amplitude coincided with a conditioning depolarization of approximately 25 mV or an absolute membrane potential of approximately −27 mV.**

tonic potential during this EJP. This reduction in the magnitude of electrotonic potentials is consistent with an increase in membrane conductance associated with the EJP.

### *Ionic Basis of the Ecitatory Junction Potential Observed Following Substance P Antagonism*

Next, we examined the possibility that the EJP observed following substance P antagonism might be due to an increase in membrane chloride conductance. Initial studies examined the effect of long-term perfusion with low-chloride solution (12.4 mmol/L) on this transient depolarization. Impalements were not maintained throughout the first 15 minutes of perfusion with low-chloride solution. However, following 15 minutes of perfusion with low-chloride solution there was a slight increase in RMP (controls = −52.2 ± 1.6 mV; low chloride = −57.3 ± 2.1 mV; n = 12 in four animals). As shown in Figure 9, following 20 minutes of perfusion with low-chloride solution, the rapid transient depolarization was markedly decreased (n = 6 in four animals). However, no significant effect was observed on the initial IJP which is known to be due to an increase in membrane potassium conductance. This selective antagonism of the EJP and not the IJP suggests that low-chloride perfusion was not abolishing the EJP through prejunctional effects on the nerve. This effect could be reversed following 30 minutes or more of perfusion with normal Krebs solution.

Subsequent studies examined the effect of the $Cl^-/HCO_3^-$ anion channel blocker DIDS on this EJP. The result of DIDS (0.6 mmol/L, >20 minutes of perfusion) was a slight increase in RMP (control = −50.7 ± 1.9 mV; DIDS = −56.3 ± 2.1 mV; $P < 0.05$, n = 12 in three animals) and a marked decrease in amplitude of the EJP. Studies with DMSO, in the same 0.1% solution used to attain dissolution of DIDS in the Krebs solution, had no effect on this EJP over a 30-minute period. Quantitative data concerning antagonism of this EJP at various conditioning hyperpolarizations with low chloride and DIDS are shown in Figure 10.

## Discussion

These studies are the first to demonstrate a nerve-mediated component of excitation in guinea pig ileum circular muscle associated with an increase in chloride conductance. Moreover, they show that this chloride-mediated excitatory response is not antagonized by atropine or substance P antagonism and is therefore due to the release of an unidentified excitatory neurotransmitter.

A number of previous studies have examined the excitatory neural pathways involved in the peristaltic reflex of the bowel. These studies have shown that

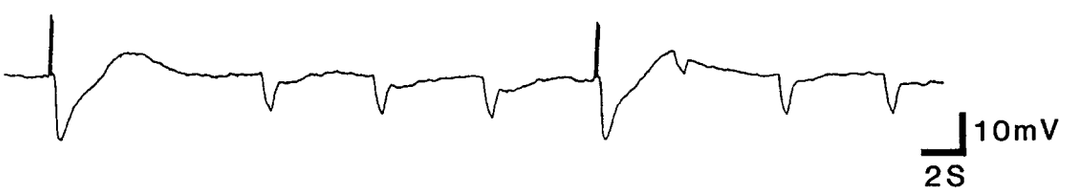


**Figure 8. Membrane resistance changes during the EJP present following substance P antagonism. The trace shows two membrane potential responses to transmural stimulation following substance P antagonism. The membrane potential responses consisted of an IJP followed by an EJP. Three brief (700 milliseconds) pulses of hyperpolarizing current of constant intensity delivered after termination of the initial transmural stimulus response resulted in electrotonic potentials having an amplitude of approximately 9 mV. A hyperpolarizing current of similar intensity delivered at the peak of the EJP resulted in an electrotonic potential having an amplitude of only 4 mV. Subsequent hyperpolarizing currents following termination of the EJP again resulted in electrotonic potentials having an amplitude of approximately 9 mV. This decrease in amplitude of the electrotonic potential during the EJP is consistent with a decrease in membrane resistance.**

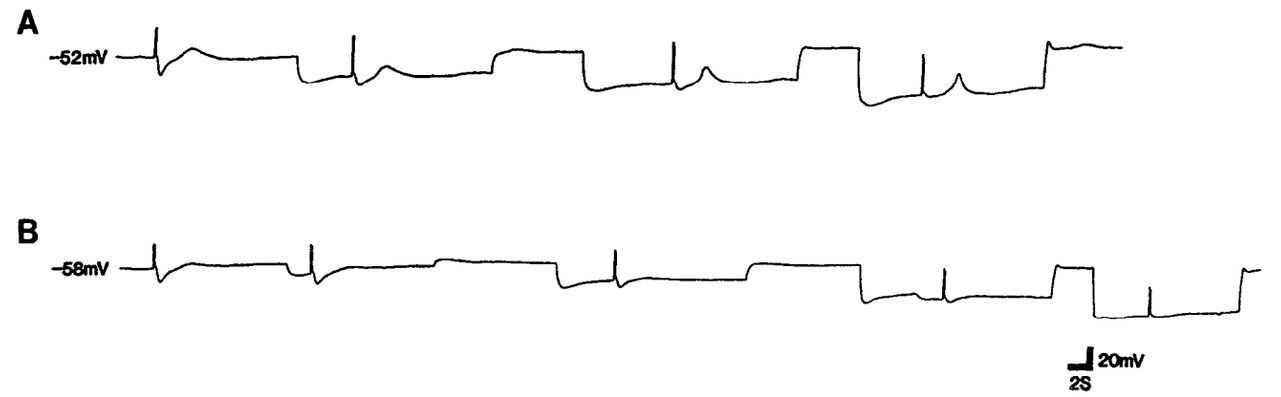


**Figure 9. Effect of low-chloride perfusion on the rapid transient depolarization observed following substance P receptor blockade with various conditioning hyperpolarizations.**

*A.* Following substance P receptor blockade, progressively greater conditioning hyperpolarizations resulted in a progressive increase in the amplitude of the rapid transient depolarization that coincided temporally with the EJP.

*B.* Following 20 minutes of perfusion with low-chloride solution, RMP had increased from −52 mV to −58 mV and the rapid transient depolarization was abolished at all conditioning hyperpolarizations.

both substance P– and acetylcholine–containing motoneurons play important excitatory roles in this reflex (5–8). However, several studies have suggested that an excitatory response is observed in guinea pig ileum following blockade of substance P and muscarinic receptors (9,10,13,14). In studies by Bywater and colleagues (12–14) of the noncholinergic membrane potential responses of guinea pig ileum circular muscle to transmural nerve stimulation, an excitatory component was shown to persist, or actually increase, after substance P antagonism. However, these investigators suggested that this response was not due to release of an excitatory neurotransmitter but to a rebound phenomenon secondary to a preceding inhibitory response. Moreover, they suggested the increase in excitation following substance P receptor blockade was due to an increase in membrane resistance (13).

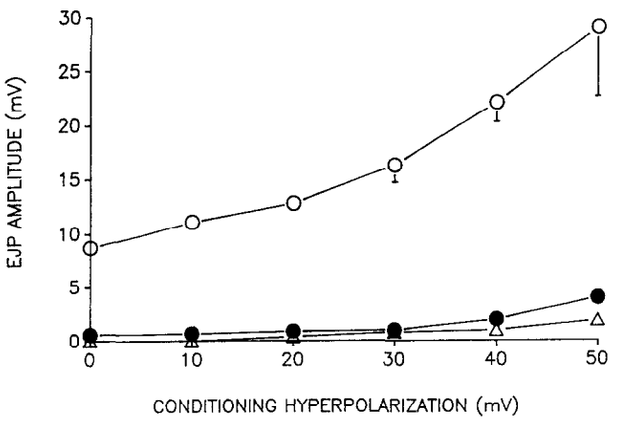


**Figure 10. Quantitative data on the amplitude of the early EJP following substance P antagonism at various conditioning hyperpolarizations (○) and the effects of low-chloride perfusion (●) and DIDS (△). The mean RMP in 16 cells in four animals was −49.3 ± 1.7 mV. The amplitude of the EJP observed following substance P antagonism progressively increased with greater conditioning hyperpolarizations. Following 20 minutes of perfusion with low-chloride solution or DIDS (600 μmol/L) a marked decrease was observed in amplitude of the EJP or rapid transient depolarization at the various conditioning hyperpolarizations ($P < 0.05$, n = 16 in four animals).**

Our studies of the noncholinergic membrane potential responses to a short train of transmural stimulation demonstrated an initial IJP followed by two EJPs referred to as early and late EJPs. Substance P antagonism had no effect on the amplitude of the initial IJP but abolished the initial portion of the early EJP and all of the late EJP. Moreover, an increase in amplitude of the latter portion of the early EJP was consistently observed following substance P antagonism. Several findings in our studies suggest that the increase in amplitude of the latter portion of the early EJP following substance P receptor blockade was not simply due to an increase in membrane resistance as previously suggested by Bywater et al. (13). These findings include the following: (a) our inability to demonstrate any change in membrane resistance following substance P antagonism with either substance P desensitization or Spantide, and (b) the absence of any increase in IJP amplitude that would be expected to be associated with any increase in membrane resistance. The basis for the increase in amplitude of the latter portion of the early EJP following substance P receptor blockade could not be determined from our studies.

Our studies examining the effect of conditioning hyperpolarizations on the nerve-mediated responses demonstrated that the IJP decreased in amplitude with progressively greater conditioning hyperpolarizations and was abolished at the estimated $E_K$ of −85 mV. This finding agrees with previous studies of the

IJP in the guinea pig ileum showing that the IJP is due to an increase in membrane potassium conductance (16–18). We also demonstrated that the initial portion of the early EJP and all of the late EJP progressively decreased in amplitude with progressively greater conditioning hyperpolarizations and were abolished at the estimated $E_K$. This abolition of the initial portion of the early EJP and all of the late EJP at $E_K$ suggests that these substance P–mediated events are due to a decrease in membrane potassium conductance.

Of particular interest was our finding that the EJP that persisted following substance P antagonism was associated with a decrease in membrane resistance. This finding suggested that this EJP might be due to an increase in membrane conductance to such ions as $Ca^{2+}$, $Cl^-$, or $Na^+$. This was supported by our finding of a progressive increase in amplitude of this EJP with progressively greater conditioning hyperpolarizations. Because the known equilibrium potential for chloride in smooth muscle is approximately −25 mV (19,20), our extrapolated reversal potential of −25 mV suggested that this event might be due to an increase in membrane chloride conductance. The abolition of the rapid transient depolarization observed during conditioning hyperpolarizations by long-term perfusion with low-chloride solution and DIDS provided additional evidence that this excitatory event was due to an increase in membrane chloride conductance. Also, DIDS is known to be a selective inhibitor of $Cl^-$/bicarbonate exchange which plays an important role in maintaining intracellular chloride concentration (20–22). Moreover, DIDS has also been reported to block movements of chloride that transfer charge (23–25). Perfusion with low-chloride solution has been shown to rapidly decrease internal chloride concentration in smooth muscle cells (19–21), thereby decreasing the driving force for efflux of chloride from the smooth muscle cell. This decrease in driving force for the efflux of chloride would result in a decrease in amplitude of any depolarizing event due to an increase in membrane chloride conductance.

Finally, our finding that the increase in amplitude of the rapid transient depolarization was associated with a decrease in amplitude of the preceding IJP at progressively greater conditioning hyperpolarization (Figures 7, 8, and 9) provides strong evidence that this excitation is not a rebound event as had previously been suggested by Bywater et al. (13).

In summary, our studies suggest the presence of noncholinergic, non–substance P motoneurons innervating the circular muscle layer of the guinea pig ileum. The nature of the excitatory neurotransmitter released from these nerves is not known, but our studies suggest that this neurotransmitter produces excitation of smooth muscle through an increase in membrane chloride conductance.

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