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Nitrergic Prejunctional Inhibition of Purinergic Neuromuscular Transmission in the Hamster Proximal Colon

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Matsuyama, Hayato, AbuBakr El-Mahmoudy, Yasutake Shimizu, and Tadashi Takewaki. Nitrergic prejunctional inhibition of purinergic neuromuscular transmission in the hamster proximal colon. *J Neurophysiol* 89: 2346–2353, 2003; 10.1152/jn.00686.2002. Neurogenic ATP and nitric oxide (NO) may play important roles in the physiological control of gastrointestinal motility. However, the interplay between purinergic and nitrergic neurons in mediating the inhibitory neurotransmission remains uncertain. This study investigated whether neurogenic NO modulates the purinergic transmission to circular smooth muscles of the hamster proximal colon. Electrical activity was recorded from circular muscle cells of the hamster proximal colon by using the microelectrode technique. Intramural nerve stimulation with a single pulse evoked a fast purinergic inhibitory junction potential (IJP) followed by a slow nitrergic IJP. The purinergic component of the second IJP evoked by paired stimulus pulses at pulse intervals between 1 and 3 s became smaller than that of the first IJP. This purinergic IJP depression could be observed at pulse intervals <3 s, but not at longer ones, and failed to occur in the presence of NO synthase inhibitor. Exogenous NO (0.3–1 μM), at which no hyperpolarization is produced, inhibited purinergic IJPs, without altering the nitrergic IJP and exogenously applied ATP-induced hyperpolarization. In the presence of both purinoceptor antagonist and nitric oxide synthase (NOS) inhibitor, intramural nerve stimulation with 5 pulses at 20 Hz evoked vasoactive intestinal peptide (VIP)-associated IJPs, suggesting that VIP component may be masked in the IJPs of the hamster proximal colon. Our results suggest that neurogenic NO may modulate the purinergic transmission to circular smooth muscles of the hamster proximal colon via a prejunctional mechanism. In addition, VIP may be involved in the neurotransmitter in the hamster proximal colon.

INTRODUCTION

Evidence from functional and morphological studies indicates that nitric oxide (NO), ATP, and vasoactive intestinal peptide (VIP) play important roles in the physiological control of gastrointestinal motility (Burnstock 2001; Crist et al. 1992; Keef et al. 1993; Kishi et al. 1996; Rae and Muir 1996). Although the inter-relationships among these mediators underlying the nonadrenergic noncholinergic (NANC) inhibitory neuromuscular transmission have been a subject of considerable debate, three possible interrelationships have been proposed. Neurogenic VIP causes relaxation through production of NO in target muscle cells of the guinea pig gastric fundus and rat colon (Dick et al. 2000; Grider 1993) and in myenteric neurons of opossum internal anal sphincter (Chakder and Rattan 1996). On the other hand, VIP release is stimulated by NO in the rat small intestine (Kurjak et al. 2001) and hamster jejunum (Matsuyama et al. 2002). ATP, which could be released from intrinsic nerves, evokes membrane hyperpolarization in circular smooth muscle cells via production of NO in postsynaptic target cells in the canine jejunum (Xue et al. 2000). To our knowledge, however, relationships between nitrergic and purinergic neuromuscular transmissions in the gastrointestinal tract have not yet been established.

Previous electrophysiological and anatomical studies have demonstrated that the hamster is a useful model for the investigation of nitrergic innervation in the gastrointestinal tract (Matsuyama et al. 1999; Toole et al. 1998). The purpose of this study was to investigate whether neurogenic NO modulates the purinergic transmission to circular smooth muscles of the hamster proximal colon. For this purpose, we used the ATP receptor antagonists, cibacron blue 3GA (CB 3GA) and suramin, and the NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME). We also examined the effects of exogenously applied ATP and NO.

METHODS

Tissue preparation

Male Syrian hamsters, 5–10 wk old, were lightly anesthetized with diethyl ether and exsanguinated via the carotid arteries, using a protocol approved by the Gifu University Animal Care and Use Committee in accordance with Japanese Department of Agriculture guidelines. Tissue preparations were similar to those previously described for hamster ileum (Matsuyama et al. 1999). Briefly, a length of about 3–4 cm of the ascending colon, which extends from the ceco-colic junction to a horse-shoe-shaped loop, was defined as the proximal colon. Whole segments, 1 cm long, were fixed to a rubber block in a 4-ml organ bath with pins and perfused with physiological salt solution (PSS) at a constant flow rate of about 3 ml/min. The composition of the PSS was as follows (in mM): 137 NaCl, 4.0 KCl, 0.5 NaH_2PO_4, 11.9 $NaHCO_3$, 2.0 $CaCl_2$, 1.0 $MgCl_2$, and 5.6 glucose. Atropine (0.1 μM), guanethidine (1 μM), and nifedipine (0.2 μM) were routinely added to the PSS. The PSS was previously oxygenated by bubbling with 95% O_2-5% CO_2 gas mixture and maintained at 32 $\pm$ 0.5°C. Tissues were allowed to equilibrate for approximately 45–60 min before experiments were undertaken.

Electrophysiological recording

Membrane potential was recorded using conventional glass microelectrodes filled with 3 M KCl with a tip resistance of 50–80 MΩ.

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The electrode was inserted into the circular muscle cells of the deep layer from the serosal side (Takewaki and Ohashi 1977). Successful insertion was confirmed when a sharp change in the voltage to a membrane potential negative to about −50 mV was observed and remained stable for ≥5 min. A pair of silver wire electrodes, one placed in the intestinal lumen and the other in the organ bath, was used for electrical field stimulation (EFS) of intramural nerves of the preparation. To record membrane potential responses to EFS, a microelectrode was inserted into a smooth muscle cell located within 2 mm of the stimulating electrode. Inhibitory junction potentials (IJPs) were evoked by EFS of intramural nerves of the tissue with square-wave pulses (1 and 5 pulses) of 0.5 ms duration at 15 V. Membrane potential changes were displayed on an oscilloscope (CS 4025, Kenwood, Tokyo, Japan). Analog electrical signals were recorded on a thermal-array recorder (RTA-1100M, Nihon Kohden, Tokyo, Japan) for illustration and analysis.

Local application of ATP

ATP was administered by a pressure application device (PV 800 Pneumatic PicoPump, World Precision Instruments). A micropipette (10 μm tip diam) filled with variable concentration of ATP (0.01 to 1.0 mM) was placed as close as possible to the recording electrode. Pressure pulses at 15 psi and 50 ms duration were used to deliver ATP. Pressure ejection of PSS did not significantly alter the membrane potential of the smooth muscle.

Preparation of NO solution

A stock solution of NO was prepared as described by Stark et al. (1991). NO gas was injected into PSS that had been deoxygenated previously by bubbling with helium for 2 h to yield a stock solution of NO ranging from 0.01 to 1.0% (vol/vol). The deoxygenated solution had no effect on the membrane potential.

Preparation of oxyhemoglobin

Oxyhemoglobin (Oxy-Hb) was prepared by a modification of the method described by Martin et al. (1984). A 10-fold molar excess of sodium dithionite ($Na_2S_2O_4$), a reducing agent, was added to a 1 mM solution of purchased hemoglobin in distilled water. Excess $Na_2S_2O_4$ was removed by dialyzing three times against 100 volumes of distilled water at 4°C. The solutions were frozen in aliquots at −20°C and stored for ≤14 days.

Drugs

L-NAME, CB 3GA, suramin, L-arginine, D-arginine, atropine, guanethidine, nifedipine, hemoglobin (bovine), VIP, VIP(6–28), and tetrodotoxin (TTX) were obtained from Sigma (St. Louis, MO). All agents were dissolved in distilled water. Stock solutions were prepared at more than 100 times higher concentrations than those used for experiments. Final concentrations of distilled water in the bathing solution were <0.01% and thus had no effect on the membrane potential. Drug concentrations given in the text were the final concentrations in the bathing solution. NO solutions were kept at 4°C and diluted to their final concentrations in PSS.

Data processing and statistical analysis

Data are expressed as mean ± SE, and *n* represents the number of experiments performed using different tissue preparations from different hamsters. When recordings were obtained from more than one cell in an individual preparation, the mean value was calculated and used. Differences between the mean values were analyzed by one-way ANOVA, followed by the Dunnett's test for multiple group comparisons, or Student's *t*-test (paired or unpaired) for comparison of two groups. *P* value <0.05 was considered significant.

RESULTS

General observations

The mean resting membrane potential recorded from the circular smooth muscle cells of the hamster proximal colon was −50.7 ± 0.5 mV ($n = 103$ cells from 20 preparations). The circular smooth muscle cells displayed small discharges of irregular frequency and amplitude (≤3 mV). EFS (0.5 ms duration, 15 V) with single pulses produced IJPs, which was comprised of two components. These IJPs were almost completely abolished by TTX (0.1 μM; $n = 7$; data not shown). The first component of the IJP (fast IJP) reached a peak value about 0.6 s after stimulation. The second slower-developing hyperpolarizing component (slow IJP) reached a peak value after about 2 s and slowly returned to the control membrane potential (Figs. 1 and 2). The latency and duration of the fast and slow IJPs are summarized in Table 1. In 84% of preparations, EFS with a single pulse evoked fast and slow components, while only the fast component of IJPs was evoked by nerve stimulation in the remaining preparations (Fig. 3*B*). In 25% of preparations, the IJPs were followed by a poststimulus depolarization, which had a peak at about 6 s after the stimulus

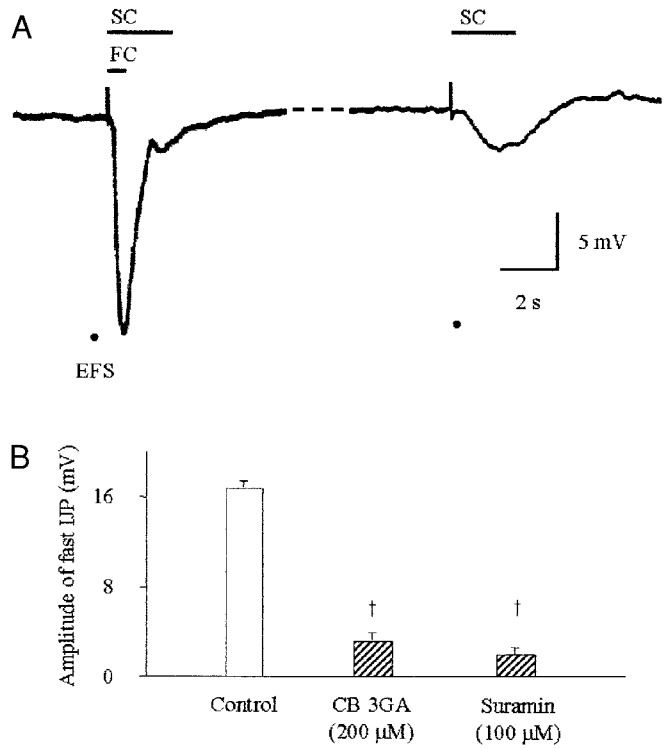

FIG. 1. Antagonizations of purinoceptor by cibacron blue 3GA (CB 3GA) and suramin inhibits fast neuromuscular transmission. *A*: responses to single pulses (0.5 ms, 15 V), delivered at dot mark, were characterized by fast followed by slow components of the inhibitory junction potentials (IJPs). Times to peak of fast and slow components of the IJPs from the stimulus are shown as the time indicated by the bar under FC and SC, respectively. The slow IJP was unchanged by CB 3GA and suramin. *B*: summary plots of the amplitude of the fast IJPs. The amplitude of fast IJP was significantly inhibited by CB 3GA and suramin. Each bar represents the mean ± SE of 8 observations. †$P < 0.01$, significantly different from the control (Dunnett's test).

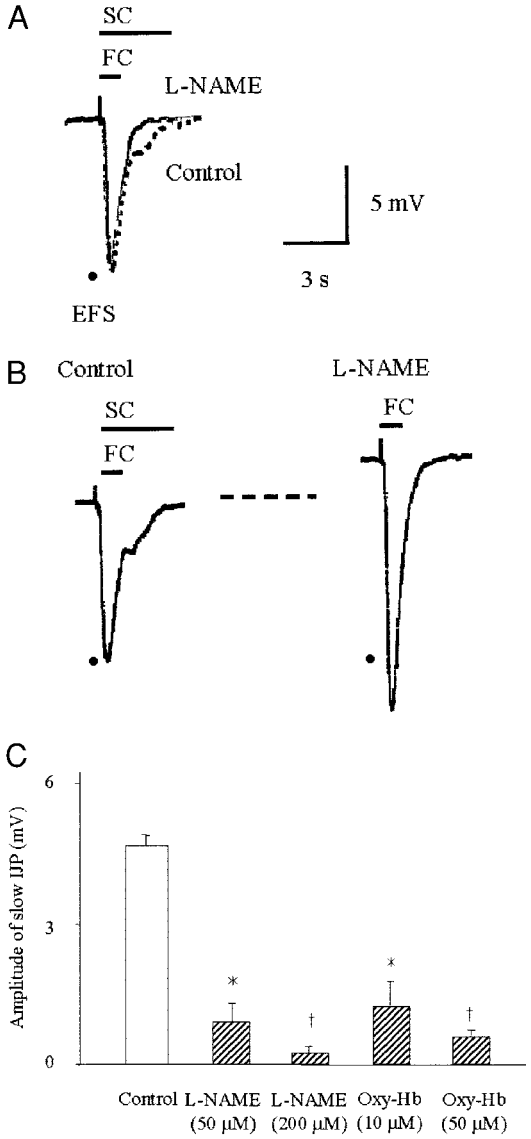

FIG. 2. Inhibition of nitric oxide synthase (NOS) and nitric oxide (NO) chelation inhibit slow neuromuscular transmission. *A*: overlays of pairs of IJPs. Times to peak of fast and slow components of the IJPs from the stimulus are shown as the time indicated by the bar under FC and SC, respectively. The fast was unchanged by N^G-nitro-L-arginine methyl ester (L-NAME) and Oxy-Hb. The dotted line in *A* shows control IJP. *B*: IJPs in response to single pulse (0.5 ms, 15 V) delivered at dot mark before and after the application of L-NAME (200 μM). L-NAME caused depolarization of membrane potential. L-NAME enhanced the fast IJP but inhibited the slow IJP. *C*: summary plots of the amplitude of slow IJPs. Amplitude of a slow IJP was significantly inhibited by L-NAME and Oxy-Hb. Each bar represents the mean ± SE of 8–9 observations. †$P < 0.01$, *$P < 0.05$; significantly different from the control (Dunnett's test).

(Fig. 3*A*). The nature of this poststimulus depolarization is not known (He and Goyal 1993).

Effects of CB3GA, suramin, L-NAME, and Oxy-Hb on IJPs

Perfusion with CB 3GA (200 μM), suramin (100 μM), L-NAME (50 μM), and Oxy-Hb (10 μM) alone, had no effect on the membrane potential ($n = 8$). However, higher concentrations of L-NAME (200 μM) and OxyHb (50 μM) induced depolarization of the membrane potential ($n = 9$; Table 2; Fig. 2*B*). Both CB 3GA and suramin inhibited the fast, but not the slow, IJPs ($n = 8$; Fig. 1). The amplitudes of the slow IJPs were 5.0 ± 0.8 and 5.0 ± 0.9 mV before and after the application of CB 3GA, respectively (paired *t*-test; $P > 0.05$; $n = 8$), and 5.0 ± 0.8 and 5.0 ± 0.9 mV for before and after the application of suramin, respectively (paired *t*-test; $P > 0.05$; $n = 8$). On the other hand, L-NAME (50 μM) and Oxy-Hb (10 μM) inhibited the slow IJPs but had no effect on the fast IJPs ($n = 8$; Fig. 2). In these experiments, the amplitudes of the fast IJPs before and after the application of L-NAME (50 μM) were 16.9 ± 0.6 and 17.2 ± 1.1 mV, respectively (paired *t*-test; $P > 0.05$; $n = 8$), and those of Oxy-Hb (50 μM) were 16.6 ± 0.5 and 17.7 ± 1.1 mV, respectively (paired *t*-test; $P > 0.05$; $n = 8$). Addition of L-NAME (200 μM) and Oxy-Hb (50 μM) enhanced the amplitude of the fast IJPs from 16.7 ± 1.1 to 22.8 ± 1.6 mV and 15.1 ± 1.1 to 21.5 ± 1.2 mV, respectively (unpaired *t*-test; $P < 0.05$; $n = 9$). The inhibition by L-NAME (200 μM) of slow IJPs was completely reversed by subsequent addition of L-arginine (5 mM) but not by its stereoisomer D-arginine (5 mM). The amplitudes of fast IJPs before the application of L-arginine and D-arginine were 14.2 ± 1.7 mV (unpaired *t*-test; $P < 0.05$; $n = 5$) and 22.0 ± 1.0 mV (paired *t*-test; $P > 0.05$; $n = 9$), respectively (paired *t*-test; $P > 0.05$; $n = 4$).

Effects of pulse intervals on IJPs

To examine if nerve excitation influences the next purinergic or nitrergic inhibitory neurotransmission, we studied the effect of conditioning pulse (0.5 ms duration, 15 V)–evoked IJPs on the test pulse (0.5 ms duration, 15 V)–evoked IJPs (test IJPs) by applying pulse intervals ranging between 0.05 and 30 s (Fig. 3). Pulse intervals ranging between 10 and 30 s had no influence on the test IJPs. On the other hand, significant depression of the fast component of the test IJPs was observed by application of pulse intervals ranging between 1 and 3 s ($n = 9$; Fig. 3*C*). Pulse intervals ranging between 0.05 and 0.5 s increased the amplitude of the fast IJP (Fig. 3*D*). EFS with two pulses delivered at 0.05-s intervals evoked fast and slow IJPs, with amplitudes of 22.1 ± 0.8 and 6.4 ± 0.5 mV, respectively ($n = 9$).

TABLE 1. *Temporal parameters of inhibitory junction potentials*

	Stimulus Condition	Control	L-NAME (200 μM)	CB 3GA (200 μM)	L-NAME + CB 3GA	VIP(6–28) (1 μM)	*n*
Latency (ms)	Single pulse	190.8 ± 2.6*†	195.8 ± 2.4*	350 ± 2.3†‡	—	190 ± 1.5*†	8
Duration (ms)	Single pulse	2896 ± 47.8*†	2351 ± 51.9*‡	2678 ± 55.9†	—	2888 ± 53.3*†	8
Latency (ms)	5 pulses at 20 Hz	207.5 ± 2.4*†	215.3 ± 2.9*	311.1 ± 2.2†‡	247.5 ± 11.3‡	200 ± 5.5*†	6
Duration (ms)	5 pulses at 20 Hz	3628 ± 31.8*†	2954 ± 59.3*‡	3555 ± 48.5†	2242 ± 43.3‡	3626 ± 26.5*†	6

Data are mean ± SE. ‡ $P < 0.01$, significantly different from the control (Dennett's test). Measurement of the latency and duration was made with reference to * fast IJP, † slow IJP, or both fast and slow IJPs. In the presence of L-NAME and CB 3GA, the latency and duration of the IJPs were measured with reference to the VIP-associated IJP.

L-NAME was used in an attempt to inhibit the neurogenic NO produced by the conditioning pulse and was anticipated to modify the purinergic component of the test pulse. Both L-NAME and Oxy-Hb reversed the depression of fast component of the test IJP ($n = 7$; Fig. 4). Higher concentrations of L-NAME (200 μM) and Oxy-Hb (50 μM) enhanced the amplitude of the fast IJPs (Fig. 4, *B* and *C*). The inhibitory effects of L-NAME (200 μM) were completely reversed by L-arginine (5 mM) but not by D-arginine (5 mM). The amplitudes of fast component of the test IJPs before and after the application of L-arginine were 2.5 ± 0.9 and 28.0 ± 1.4 mV, respectively (unpaired *t*-test; $P < 0.05$; $n = 5$). The amplitudes of the fast component of the test IJPs before and after the application of D-arginine were 2.4 ± 0.8 and 2.3 ± 1.0 mV, respectively (paired *t*-test; $P > 0.05$; $n = 4$). These results indicate that endogenous NO may inhibit purinergic IJPs at pulse intervals more than 0.05s.

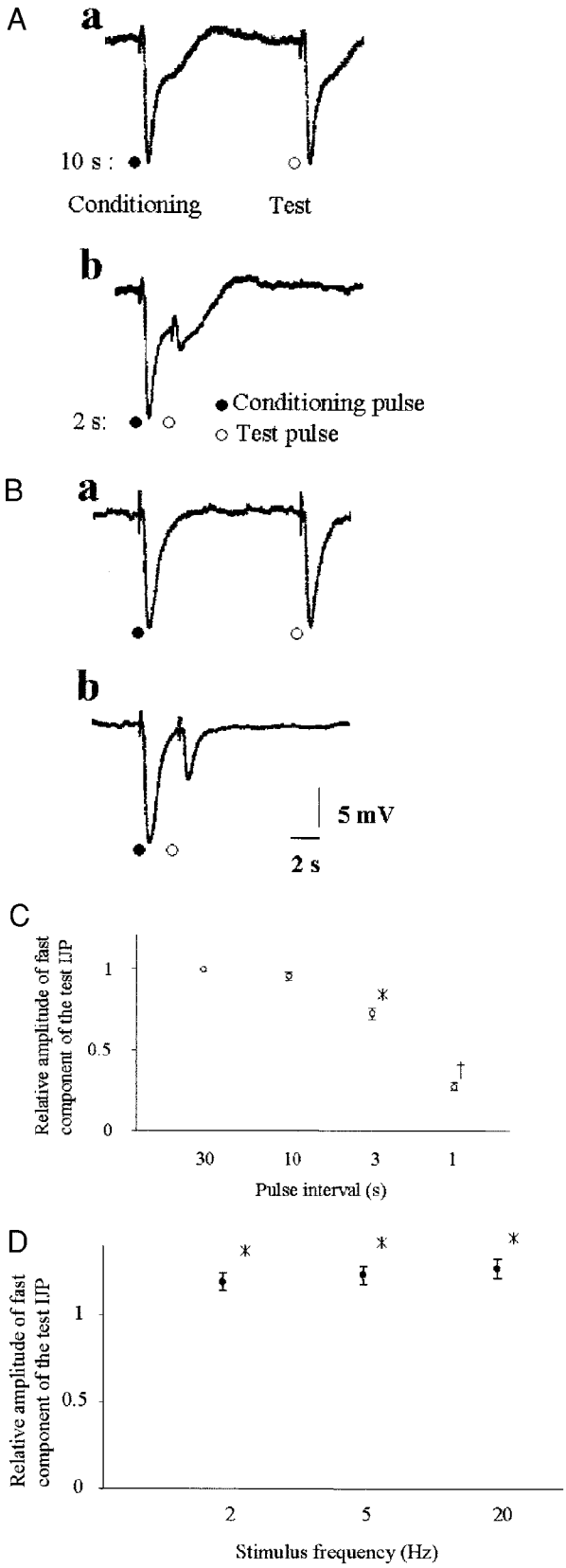

FIG. 3. Depression of purinergic transmission by endogenous NO. *A* and *B*: IJPs in response to single pulse (0.5 ms, 15 V), delivered at dot mark, delivered every 2 s (*Ab* and *Bb*) and 10 s (*Aa* and *Ba*). The fast IJP was inhibited by 2-s stimulus intervals but not by 10-s stimulus intervals. Solid and open circles represent conditioning and test pulses, respectively. *A* and *B* were recorded from separate preparations. *B*: slow IJP was not detected in this preparation. *C* and *D*: summary plot of the relative amplitudes of test IJPs. Amplitude of the fast component of test IJPs was significantly inhibited by endogenous NO when paired pulse stimulation was delivered with an interpulse interval of 1–3 s, but not at pulse intervals ranging between 10 and 30 s. However, pulse intervals ranging between 0.05 s (20 Hz) and 0.5 s (2 Hz) increased the amplitude of the fast IJPs. †$P < 0.01$, *$P < 0.05$; significantly different from the respective control (Dunnett's test).

TABLE 2. *Effects of CB 3GA, suramin, Oxy-Hb, and L-NAME on membrane potential*

	Membrane Potential (mV)	*n*
Control	−53.7 ± 0.7	14
CB 3GA (200 μM)	−54.8 ± 0.5	8
Suramin (100 μM)	−54.1 ± 0.8	8
L-NAME (50 μM)	−50.5 ± 0.9	8
L-NAME (200 μM)	−45.7 ± 0.9*	9
Oxy-Hb (10 μM)	−51.9 ± 0.7	8
Oxy-Hb (50 μM)	−47.2 ± 0.7*	9
VIP(6–28) (1 μM)	−54.0 ± 0.8	7

Data are mean ± SE. * $P < 0.05$, significantly different from the control (Dunnett's test).

Effect of exogenous NO on IJP and ATP-induced hyperpolarization

Application of exogenous NO at various concentrations ranging from 0.3 to 1 μM inhibited the fast test IJPs; however, nitrergic IJP was not altered ($n = 8$). There were no significant alterations in the membrane potential of the smooth muscle ($n = 10$) by exogenously applied NO (0.3–1 μM). Higher concentrations of NO (3–10 μM) induced a concentration-dependent increase in hyperpolarization (Fig. 5). Repetitive (3-s intervals) pressure pulses of exogenous ATP produced hyperpolarizations. The amplitudes of the first and second ATP-induced hyperpolarizations were constant. The ATP-induced hyperpolarizations were not significantly altered by exogenously applied NO (1 μM; Fig. 6). The amplitudes of ATP (1 mM)-induced hyperpolarizations in the absence and presence of the exogenous NO (1 μM) were 10.2 ± 0.7 and 10.1 ± 0.8 mV, respectively (paired *t*-test; $P > 0.05$; $n = 6$).

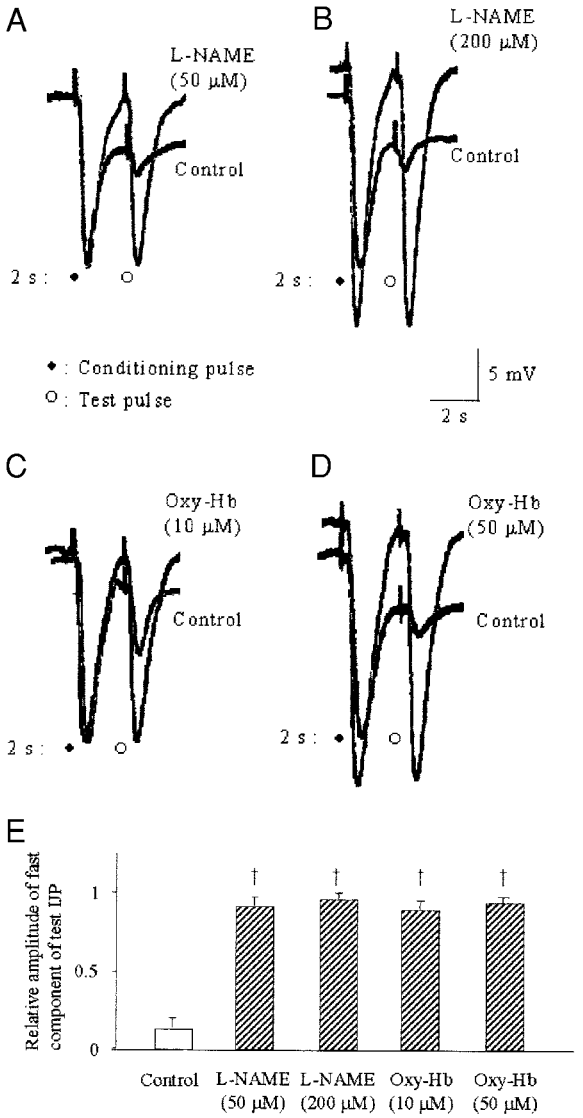

FIG. 4. Effects of L-NAME and Oxy-Hb on purinergic IJP depression. *A–D*: overlays of pairs of IJPs in response to single pulse (0.5 ms, 15 V), delivered at dot mark, delivered every 2 s. Solid and open circles represent conditioning and test pulses, respectively. *E*: summary plot of the amplitudes of IJPs. L-NAME and Oxy-Hb significantly reduced the purinergic IJP depression. Each bar represents the mean ± SE of 7 observations. †$P < 0.01$ significantly different from the respective control (Dunnett's test).

Effect of VIP(6–28) on IJPs and VIP-induced hyperpolarizations

To investigate whether peptidergic transmission contributes to the IJP generation, we tested the effect of VIP receptor antagonist, VIP(6–28), on IJPs evoked by EFS (0.5 ms duration, 15 V) with single pulses and trains of five pulses. Exogenously applied VIP (0.3–3 μM) induced a concentration-dependent increase in hyperpolarization, which was inhibited by the application of VIP(6–28) (1 μM). The amplitude of VIP (3 μM)-induced hyperpolarization was 6.5 ± 0.7 and 1.2 ± 0.4 mV before and after the application of VIP(6–28), respectively (unpaired *t*-test; $P < 0.05$; $n = 6$). Addition of VIP(6–28) had

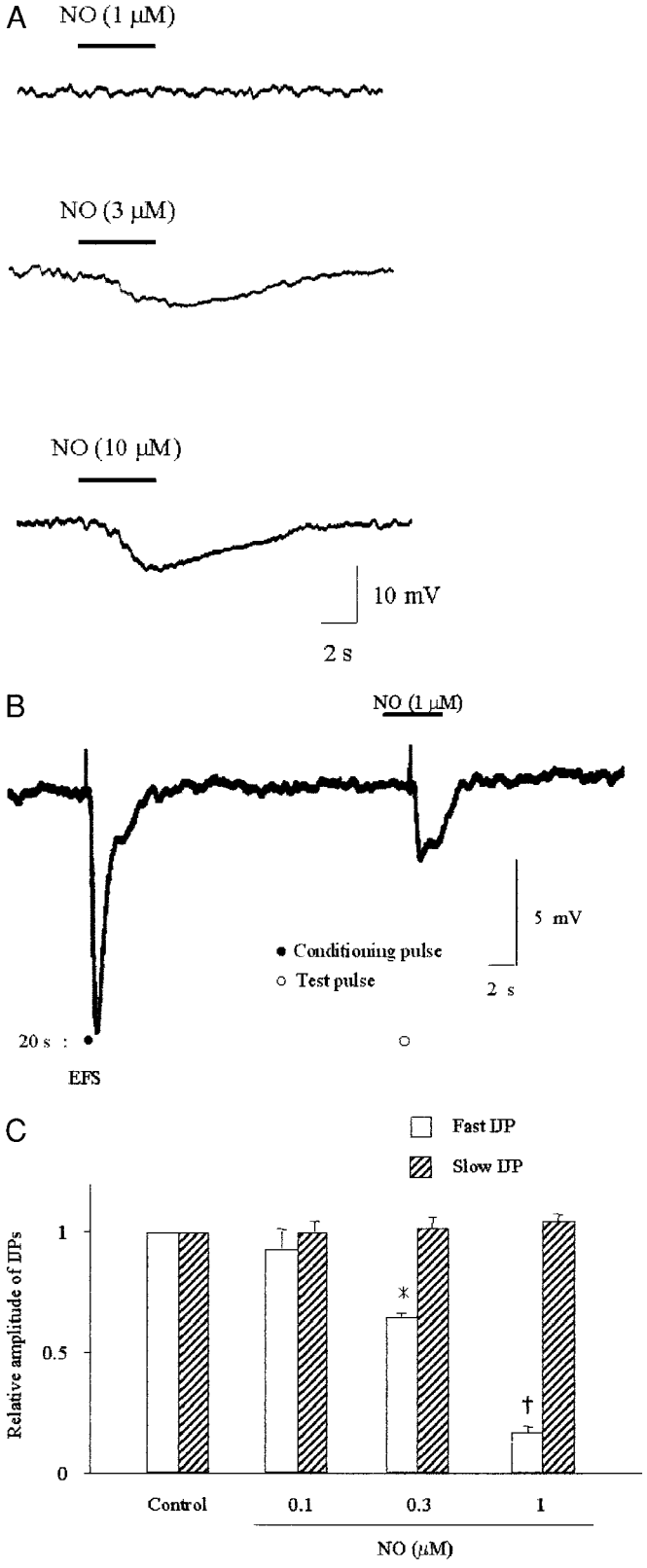

FIG. 5. Effect of exogenously applied NO on membrane potential and IJPs. *A*: NO caused a concentration-dependent hyperpolarization. *B*: IJPs in response to single pulses (0.5 ms, 15 V), delivered at dot mark, derived every 20 s. NO was applied at the time indicated by the bar. IJP during application of NO was defined as the test IJP. *C*: summary plot of the amplitudes of the test IJPs. Exogenously applied NO significantly inhibits purinergic IJPs but did not alter nitrergic IJPs. Each represents the mean ± SE of 8 observations. †$P < 0.01$, *$P < 0.05$; significantly different from the respective control (Dunnett's test).

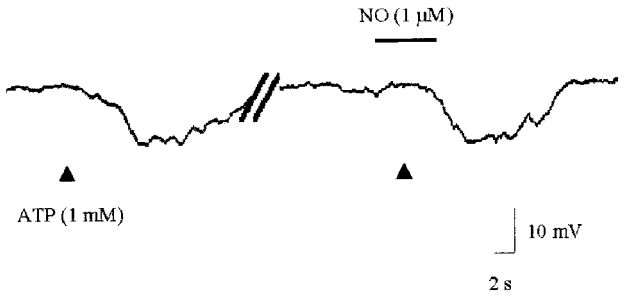

FIG. 6. Effect of exogenously applied NO on the ATP-induced hyperpolarization. Pressure application of ATP induced hyperpolarization. NO was applied at the time indicated by the bar. NO did not alter the ATP-induced hyperpolarization.

no effect on the membrane potential (Table 2), and both components of single pulse stimulation evoked IJPs. The amplitudes of fast and slow components of the single pulse–evoked IJPs were 17.1 ± 0.7 and 5.0 ± 0.4 mV, respectively, in control, and 16.8 ± 0.6 and 4.9 ± 0.3 mV, respectively, in the presence of VIP(6–28) (paired *t*-test; $P > 0.05$; $n = 7$). On the other hand, VIP(6–28) significantly inhibited the CB 3GA- and L-NAME–resistant IJPs evoked by trains of five pulses at 20 Hz (Fig. 7).

DISCUSSION

The results of this study suggest that neurogenic NO modulates purinergic transmission from enteric neuron to the circular muscles of the hamster proximal colon via a prejunctional mechanism. Our evidence in support of this conclusion can be summarized as follows: *1*) L-NAME, a nitric oxide synthase (NOS) inhibitor, reversed the purinergic IJP-depression produced by the second stimulus of paired stimuli delivered with pulse intervals ranging between 1 and 3 s; *2*) exogenously applied NO inhibited purinergic IJP; and *3*) neither the first nor second ATP-induced hyperpolarization was affected by exogenous NO. Although ATP and NO have been widely recognized as mediators for NANC inhibitory neurotransmissions (Boeckxstaens et al. 1993; Burnstock 2001; Crist et al. 1992; Jenkinson and Reid 2000; Rae and Muir 1996; Smits and Lefebvre 1996; Toole et al. 1998; Xue et al. 1999, 2000), such an inhibitory action of neurogenic NO on the purinergic transmission, has not been demonstrated previously in the gastrointestinal tract. Our data confirm that both endogenously and exogenously applied NO inhibited purinergic IJPs but did not have significant effect on exogenously applied ATP-induced hyperpolarizations. These observations may explain the prejunctional effects of NO on the purinergic transmissions.

In the present study, nitrergic IJPs lasted about 3 s after EFS, indicating that the effect of neurogenic NO can be maintained for about 3 s after stimulation. When the test pulse was applied 1–3 s after the conditioning pulse, the amplitude of the purinergic IJP evoked by the test pulse was significantly smaller than that evoked by the conditioning. In support of these observations, it has been demonstrated that the decay rate of NO has a half-life ranging between 0.5 and 5 s (Lancaster 1994). Furthermore, when NANC nerve stimulations were applied at a pulse interval ranging between 0.5 and 2 s, NANC relaxations were completely inhibited by a NOS inhibitor in the canine fundus (Bayguinov et al. 1999). Thus stimulus intervals shorter than 3 s may enable neurogenic NO to regulate the purinergic transmission in the hamster proximal colon.

This study emphasizes the interplay between purinergic transmission and spontaneously released NO in mediating the inhibitory neurotransmission underlying IJPs. L-NAME significantly altered the purinergic component of the single pulse-evoked IJP, indicating that intrinsic, spontaneously released NO may affect the purinergic transmission to circular smooth muscle cells of the hamster proximal colon. The hypothesis is consistent with the observation that tonic release of NO regulated the spontaneous electrical and contractile activities of the canine colon (Keef et al. 1997) and that L-NAME caused depolarization of resting potential in the canine ileocolonic sphincter (Ward et al. 1992). However, the inhibitory role of spontaneously released NO on the purinergic neuromuscular transmission has not been reported previously.

In this study, two pulses of EFS delivered at 2–20 Hz enhanced summation of the purinergic and nitrergic components of the IJPs. The nitrergic component of the IJPs induced by two pulses at 2–20 Hz was unaffected by CB 3GA. These results suggest that the nitrergic component is not inhibited but enhanced by either neurogenic ATP or stimulus condition. Consistent with this observation, ATP was proposed to cause hyperpolarization via NO production in postsynaptic target cells in the canine jejunum (Xue et al. 2000). Thus ATP might enhance nitrergic neuromuscular transmission in the hamster proximal colon. Another possibility is that EFS with two pulses at 2–20 Hz might produce summation of nitrergic IJPs in the hamster proximal colon. In the rat colon, EFS with two pulses at 10 Hz produced summation of IJPs (Kishi et al. 1996). Further studies are necessary to confirm the above two possibilities.

Previous studies suggested that neuronal ATP and NO may contribute to IJPs and thus regulate gastrointestinal motility in

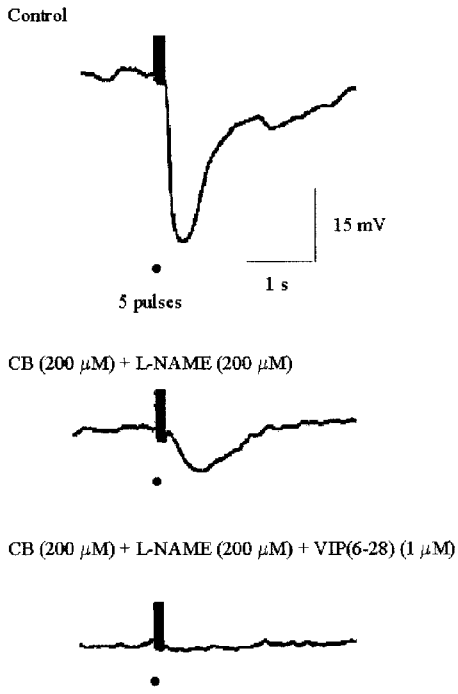

FIG. 7. Effect of vasoactive intestinal peptide [VIP(6–28)] on the CB 3GA- and L-NAME–resistant IJPs. IJPs are evoked by trains of 5 pulses. Note that trains of 5 pulses evoked ATP- and NO-insensitive IJPs.

the guinea pig taenia coli (Den Hertog et al. 1985) and opossum esophagus (Conklin et al. 1995). L-NAME inhibited the IJPs and relaxations in the canine small intestine (Stark et al. 1991) and canine fundus (Bayguinov et al. 1999). On the other hand, L-NAME reduced the threshold volume required to trigger emptying of the guinea pig small intestine (Waterman and Costa 1994). Another inhibitor of NOS, N^G-monomethyl-L-arginine, inhibited the increase rate of transient lower esophageal sphincter relaxations induced by gastric distension in human (Hirsch et al. 1998). Endogenous and exogenous ATP inhibited intestinal peristalsis in the guinea pig small intestine (Heinemann et al. 1999). L-NAME and a P2-purinoceptor antagonist, suramin, attenuated relaxations of the antrum in anesthetized rats (Glasgow et al. 1998). Furthermore, electrical and reflex stimulation of enteric nerves produce NO-associated IJPs in the guinea pig colon (Watson et al. 1996). These findings indicate that purinergic and nitrergic IJPs might underlie the peristaltic reflex. Furthermore, the inhibition of NOS activity shortened a latency of peristaltic wave initiation and increased a velocity of propulsion of an intraluminally distended balloon in the rabbit distal colon (Ciccocioppo et al. 1994). The existence of NOS and ATP was found in the myenteric plexus of rat ileum and colon (Belai and Burnstock 1994) and human stomach and small intestine (Belai and Burnstock 2000), suggesting that NO and ATP may be released from the nerves. Thus it is likely that neurogenic NO-induced inhibition of purinergic transmission might be involved in the mechanism that regulates gastrointestinal peristaltic movement.

The enteric nervous system has been mapped for ATP, NOS, and VIP by immunohistochemistry. These studies showed colocalization of nitrergic neurons and either ATP or VIP in myenteric neurons of the rat ileum and colon (Belai and Burnstock 1994) and human colon (Porter et al. 1997). Furthermore, previous studies demonstrated that NO can stimulate VIP release from the isolated myenteric plexus of the guinea pig ileum (Grider and Jin 1993) and rat small intestine (Kurjak et al. 2001), suggesting a possible presynaptic stimulating role for NO on VIP release. Thus evidence based on the overlapping for NOS and VIP suggests the existence of interaction between these neurotransmitters in mediating the inhibitory neurotransmission. In the myenteric plexus of the rat ileum and colon (Belai and Burnstock 1994) and human stomach and small intestine (Belai and Burnstock 2000), ATP and NOS are colocalized. Although the interrelationship between neurogenic ATP and NO in mediating the inhibitory neurotransmission is not completely understood, our data provide strong support for further relationship between them.

Paired pulse stimulation of the nerves from 0.05 to 0.5 s may evoke facilitation of action potential in purinergic nerves (Landfield et al. 1986) and thus give rise to an enhancement of evoked neurotransmitter release in the hamster proximal colon. These data consistent with the observations that elevation of number of the action potential on the nerve axons or close to the release sites resulted in depolarization of membrane potential and enhancement of neurotransmitter release (Khakh and Henderson 2000). Endogenous ATP has acted on presynaptic P2X receptors to enhance the amount of neurotransmitter released (LePard et al. 1997). These observations may explain why there is an increase in the amplitude of the fast IJP in the hamster proximal colon.

This study revealed that VIP was involved in the IJPs evoked by trains of five pulses at 20 Hz but not in the IJPs evoked by single pulse stimulation in the hamster proximal colon. Therefore we speculate that stronger stimulus strength is needed to add the VIP component to the IJPs in the hamster proximal colon. This hypothesis is consistent with the following observations: *1*) single pulse stimulation resulted in an ATP-mediated IJP, which was unaffected by VIP receptor antagonist, but trains of pulses resulted in VIP-mediated IJP in the guinea pig ileum (Crist et al. 1992) and *2*) trains of pulses evoked VIP-associated IJPs in the hamster jejunum (Matsuyama et al. 2002).

In conclusion, we have demonstrated in the present study that neurogenic NO seems to modulate the purinergic transmission to the circular smooth muscle of the hamster proximal colon via a prejunctional mechanism. The results obtained with pharmacological, electrophysiological, and morphological studies support the roles of ATP and NO as important NANC inhibitory neurotransmitters in the hamster proximal colon.

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