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NANC inhibitory neuromuscular transmission in the hamster distal colon

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Abstract

The neurotransmitter(s) that generate the inhibitory junctional potential (IJP) in the circular muscle of hamster distal colon and their mechanisms have not been elucidated. The aim of the present study, therefore, was to determine the contributing roles of the non-adrenergic, non-cholinergic (NANC) inhibitory transmitter(s) including nitric oxide (NO), adenosine 5′-triphosphate (ATP) and vasoactive intestinal polypeptide (VIP) in the generation of IJP in the hamster distal colon. For this purpose, the effects of the corresponding blockers of these putative NANC inhibitory mediators have been investigated using microelectrode technique. Intracellular membrane potential recordings were made from smooth muscle cells at 35 °C in Tyrode's solution that contained atropine (0.5 μM), guanethidine (3 μM) and nifedipine (0.5 μM). Single electrical stimuli (0.5 ms, 50 V) as well as trains of two and five pulses (20 Hz at the same duration and voltage) elicited NANC IJP consisted of initial fast (IJP-F) followed by a slow hyperpolarization (IJP-S). The response had been abolished by tetrodotoxin (TTX, 0.3 μM). The nitric oxide synthase (NOS) inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 200 μM) blocked IJP-S but enhanced IJP-F. The later had been blocked with suramin, a universal P2 receptor antagonist, or with CBF3GA, a P2Y receptor antagonist at dose-dependent fashions. The IJP-F had been markedly inhibited by desensitization of P2Y receptor with its putative agonist 2-methylthio-ATP (2-meSATP, 50 μM for 30 min). IJP-F was sensitive to the P2Y1 receptor specific antagonist A3P5PS (10 μM) and to the G-protein inhibitor, pertussis toxin (PTX, 400 ng/ml for 2 h) as well as to the small and intermediate Ca^{2+} sensitive K^+ channels blocker, apamin (0.3 μM). IJP-S was blocked by the guanylate cyclase (GC) inhibitor, 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ, 10 μM) and was partially sensitive to apamin. Exogenously applied ATP (100 μM–1 mM) produced typical hyperpolarization that was blocked by suramin, CBF3GA and 2-meSATP desensitization; while exogenously applied NO (3–10 μM) produced slowly developing hyperpolarization that was not blocked by L-NAME but ODQ. In the presence of both purinergic and nitrergic inhibitors, stimulation using a train of eight pulses at 25 Hz evoked a small slow hyperpolarization that was sensitive to the VIP antagonist (VIP 6–28, 1 μM). Exogenous application of VIP (1–10 μM) produced similar response that was not evident in the presence of VIP 6–28. These data indicate that NANC IJP that is generated in the circular muscle cells of hamster distal colon is mediated by ATP and NO via P2Y1/P2Y2 receptor and GC-dependent pathways, respectively. A masked role for VIP is also indicated.

Keywords: Colon; Non-adrenergic; Non-cholinergic; Inhibitory junctional potential; ATP; Nitric oxide; Vasoactive intestinal polypeptide

1. Introduction

Accumulated evidence indicates that the mediator(s) of inhibitory control is not uniform throughout the gastrointestinal tract, but is quite variable from region to region. In addition to the regional difference, there is a difference among species. Adenosine triphosphate (ATP), nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) are the main inhibitory mediators and all are being released from inhibitory nerves in varying combinations, depending on the species and region of the gastrointestinal tract [1,2].

In the large intestine, although ATP was reported to participate in the non-adrenergic, non-cholinergic (NANC) relaxation of the guinea pig colon [3], there is evidence against the role of purines as NANC transmitters in the rat colon [4]. In addition to the role of ATP, NO had been reported to be an inhibitory mediator in the mid and distal portions of rat's colon [5,6]. However, that role of NO was not evident at the longitudinal muscle of the distal portion [7,8]. Furthermore, although VIP was suggested as a mediator of NANC relaxation in the colon of rats [9,7] it does not have any role in the NANC relaxation in mouse colon [10] and in canine ileocolonic junction [11]. In the mouse distal

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colon, the fast IJP was found to be purinergic in nature and could be modulated with PACAP [12]. Coordination between the transmitters mediating the inhibitory response may have functional implications in regulating intestinal peristalsis.

Compared to other species, little information is available in literature about the identification of NANC neurotransmitters in the hamster gastrointestinal tract, particularly large intestine. Also, information about the neuroeffector mechanisms is scarce. In a previous report [13], our group has studied the interrelationship between the mediators of the IJP of hamster proximal colon and concluded that the neurogenic NO inhibits the purinergic transmission to its circular smooth muscle via a prejunctional mechanism. Taking in consideration that there are regional differences in mediators of IJP, this study, therefore, has been focused on the identification of the neurotransmitters and the underlying neuroeffector mechanisms mediating the IJP of the distal portion of the hamster colon. For this purpose, the specific antagonists of the above mentioned putative transmitters have been used.

2. Materials and methods

2.1. Tissue preparation

Tissues were obtained from male Syrian hamsters aging 6–8 weeks and weighing 80–120 g. Under light ether anaesthesia, hamsters were killed by exsanguination of the carotid artery. Through an abdominal incision, a length of about 3–4 cm of intact distal colon was removed and immediately immersed in physiological salt solution (PSS; see below) at room temperature. The colonic segment was placed in a dissection dish containing PSS and the intraluminal contents were flushed using a small cannula filled with PSS.

The surgical procedures and pre- and post-operative care of the animals conformed to the Gifu University Animal care and Use committee in accordance with Japanese Department of Agriculture guidelines and all efforts were made to minimize animal suffering and to reduce the number of animal used.

2.2. Electrophysiological recordings

One centimeter-length of the colonic segment was excised and pinned to the rubber floor of the experimental chamber of an organ bath of 5 ml capacity. The bath was constantly perfused with pre-warmed (35 °C) PSS containing 0.5 μM atropine, 3 μM guanethidine and 0.5 μM nifedipine at a flow rate of about 3 ml/min. The PSS was previously bubbled with 95% O_2:5% CO_2 gas mixture. Tissue preparations were allowed to equilibrate for approximately 1 h before experiments were undertaken.

Membrane potential changes were recorded using conventional glass microelectrodes that had resistances of 50–80 MΩ when filled with 3 M KCl. The microelectrode insertions were made into the circular muscle cells of the deep layer through the serrosal side [14]. A successful insertion was confirmed when a sharp drop in the recorded voltage to a negative resting membrane potential of about −50 mV was established and remained stable. Application of electrical field stimulation (EFS) to the intramural nerves of the preparation was done by a pair of silver wire electrodes. The two electrodes were placed parallel to the longitudinal axis of the preparation so that one is passing through the lumen of the fixed tissue while the other wire outside the preparation in the PSS of the bath. The two electrodes were connected to square-wave stimulator (Sen-2201, Nihon Koden, Tokyo, Japan).

Membrane potentials in response to EFS were recorded from circular smooth muscle cells located within 2 mm of the simulating electrode (inside the lumen). Inhibitory junction potentials (IJPs) were evoked by EFS of intramural nerves of the preparation with square-wave pulses (one to five pulses) of 0.5 ms duration at 50 V. Membrane potential changes were displayed on an oscilloscope (CS 4025, Kenwood, Tokyo, Japan). Analogue electrical signals were recorded on a thermal-array recorder (RTA-1100M, Nihon Kohden, Tokyo, Japan) for illustration and further analysis.

2.3. Physiological solutions

The physiological solutions used in this study had the following composition (mM): NaCl 137, KCl 4.0, $Na_2H_2Po_4$ 0.5, $NaHCo_3$ 11.9, $CaCl_2$ 2.0, $MgCl_2$ 1.0 and glucose 5.6. The pH of the solution was 7.4.

2.4. Drugs

The drugs used were as follows: N^G-nitro-L-arginine methyl ester (L-NAME), guanethidine sulphate, nifedipine, vasoactive intestinal polypeptide (VIP), VIP(6–28), ATP, 1H-[1,2,4] oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ), 2-methylthio-ATP (2-meSATP), pertussis toxin (PTX), apamin, adenosine-3′-phosphate-5′-phosphosulfate (A3P5PS) and tetrodotoxin (TTX) (Sigma, St. Louis, MO, USA); suramin sodium, atropine sulphate monohydrate (Wako Pure Chemical Industries, Osaka, Japan); 1-amino-4-[[4-[[4-chloro-6-[[3(or 4)-sulphophenyl]-amino-1,3,5-triazin-2-yl]amino]-3-sulphophenyl]amino-9,10-dihydro-9,10-dioxo-2-anthracenesulphonic acid (Cibacron blue F3GA; CB F3GA) (Funakoshi, Tokyo, Japan).

2.5. Preparation of nitric oxide solution

A stock solution of NO was prepared by a modification of the method of [15]. Briefly, NO gas was injected into PSS which was previously deoxygenated by gassing with helium for 2 h, to give a stock solution of NO 1% (v/v).

2.6. Local application of ATP

ATP was added by a pressure application device, where a micropipette (10 μm tip diameter) was filled with ATP solution (100 μM–1 mM) and adjusted as close as possible to the recording microelectrode. Pressure pulses were delivered at 15 psi and 50 ms duration.

Table 1
Table showing electrophysiological parameters of IJPs evoked by EFS (each value is mean ± S.E. mean of 15 preparations)

Stimulus condition	Latency (ms)	Amplitude (mV) IJP-F UPS		Duration (ms)
Single pulse	195.8 ± 4.2	18.4 ± 1.8	3.3 ± 0.3	2995 ± 51.8
Double pulse	207.5 ± 3.9	22.1 ± 2.1	5.3 ± 0.4	3472 ± 57.1
Five pulses	215.5 ± 4.8	27.3 ± 3.2	8.3 ± 0.9	3811 ± 55.2

2.7. Data presentation and statistical analysis

Data are expressed as mean ± S.E. "*n*" in Section 3 refers to number of animal preparations on which observations were made. Differences between the means were analyzed by Student's *t*-test (paired or unpaired) for comparison of two groups. *P* value of less than 0.05 was considered significant.

3. Results

3.1. General

The resting membrane potential of the smooth muscle cells of hamster distal colon was 50 ± 2.4 mV ($n = 75$). When unstimulated, the smooth muscle cells in the colonic tissue were either electrically quiescent or showing slow waves with small amplitudes not more than 2 mV and frequency of 7 ± 1.5 cycles/min. Intramural nerve stimulation evoked IJPs which were abolished by TTX (0.3 μM, $n = 3$, data not shown). Each IJP consisted of two components, initial fast (IJP-F) followed by slow hyperpolarization (IJP-S). As shown in Fig. 1 and Table 1, the amplitudes of the IJPs were graded depending on the stimulus strength; with a single pulse, the amplitudes of IJP-F and IJP-S were 18.4 ± 1.8 and 3.3 ± 0.3, respectively ($n = 15$). The IJPs had a latency of 195 ± 4.2 and a total duration of 2995 ± 51.8 ms; these values increased gradually depending on the strength of the EFS. Table 1 summarizes the electrophysiological parameters of IJPs after single, double and five-pulse stimulations.

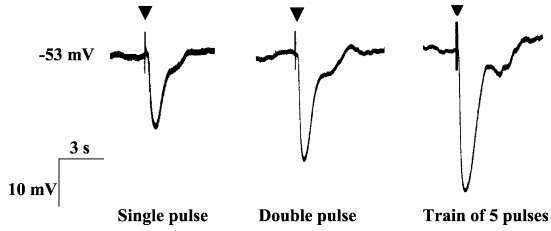

Fig. 1. Intracellular recording of electrical activity of circular smooth muscle of hamster distal colon in response to EFS delivered at (▼) (single, double and train of five pulses each 0.5 ms at 20 Hz) in presence of atropine (0.5 μM), guanethidine (3 μM). NANC IJP consisted of initial fast (IJP-F) followed by slow hyperpolarization (IJP-S).

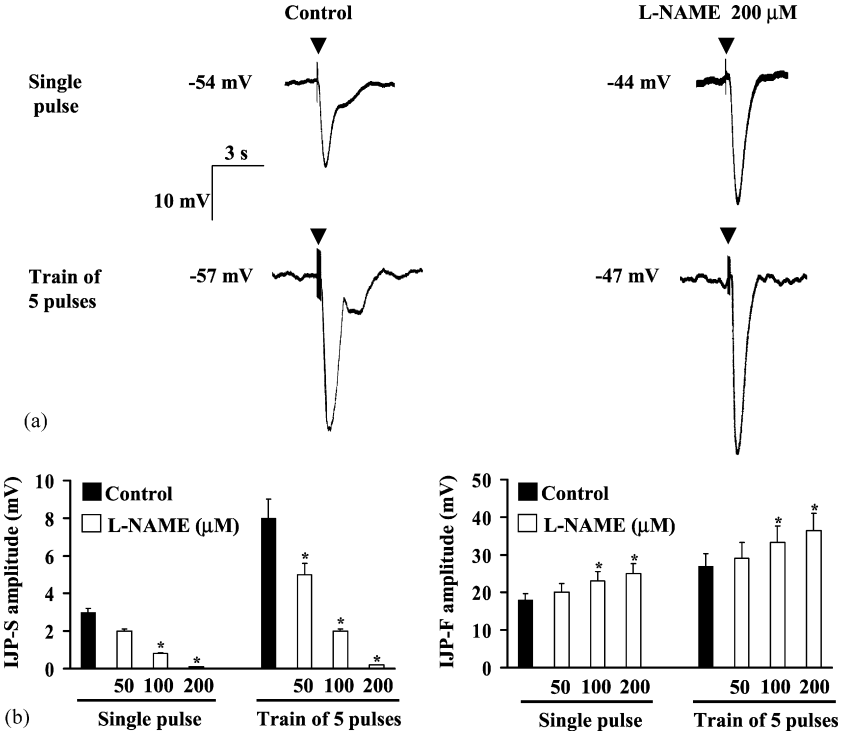

Fig. 2. Typical tracings (a) and histogram (b) showing the effects of N^G-Nitro-L-arginine methyl ester (L-NAME; 50–200 μM) on the junction potentials evoked by EFS (0.5 ms, 50 V, single pulse and a train of five pulses at 20 Hz), applied at (▼) in the circular smooth muscle of hamster distal colon. The effect of the drug was recorded after 20 min of contact. L-NAME blocked only IJP-S. Each value is mean ± S.E. mean of 12 preparations. Significantly different from control values: $^*P < 0.05$.

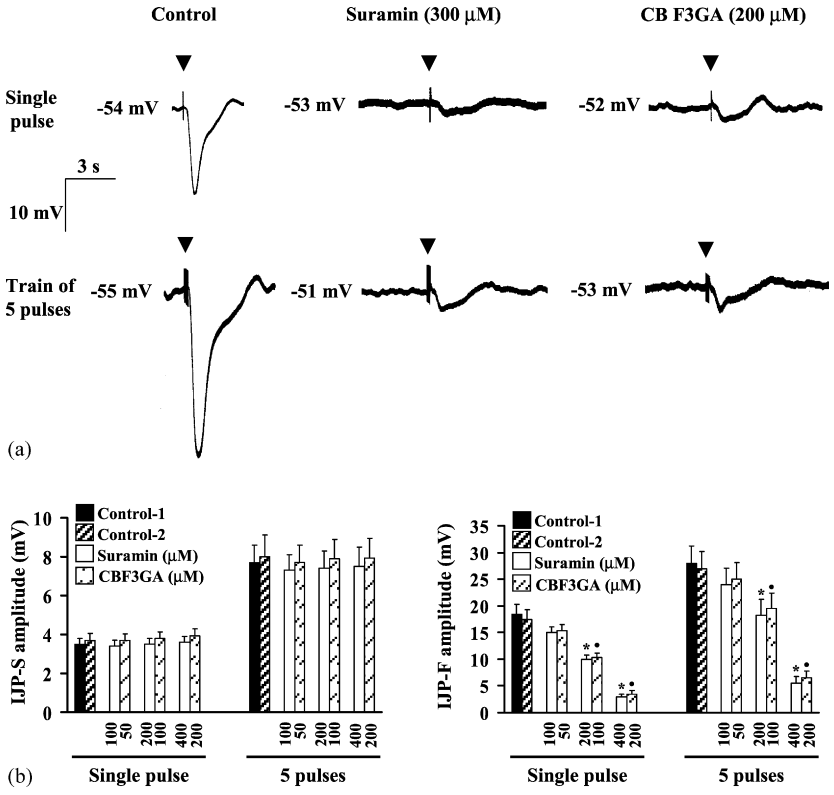

Fig. 3. Typical tracings (a) and histogram (b) showing the effect of the universal P2 receptor antagonist, suramin (100–400 μM) and the universal P2Y receptor antagonist CB F3GA (50–200 μM) on junctional potentials evoked by EFS (0.5 ms, 50 V, single pulse and a train of five pulses at 20 Hz, applied at (▼) in the circular smooth muscle of hamster distal colon. Both suramin and CB F3GA markedly inhibited IJP-F without any effects on IJP-S. Each value is mean ± S.E. mean of three (suramin) or five (CB F3GA) preparations. Significantly different from control values: $^{*}P < 0.05$.

3.2. Effect of L-NAME on IJPs evoked by intramural nerve stimulation

The mean amplitudes of IJPs (fast/slow) evoked by single stimulus and a train of five pulses were 18.7 ± 1.9 mV/3.5 ± 0.3 mV and 27.8 ± 3.5 mV/8.6 ± 0.8 mV, respectively ($n = 12$). Application of L-NAME, a neuronal NO synthase inhibitor, dose-dependently depolarized the resting membrane potential and inhibited IJP-S. Two hundred micromolar of L-NAME depolarized the resting membrane potential by about 10 mV and completely abolished the IJP-S that was produced by either single or five-pulse stimuli. The amplitudes of IJP-F had been significantly increased from 17.5 ± 1.7 mV/27.3 ± 2.9 mV to 22 ± 2.1 mV/33.5 ± 3.2 mV after single and five-pulse stimuli, respectively (Fig. 2). The inhibition of IJP-S by L-NAME was completely reversed by subsequent addition of L-arginine (3 mM) but not by its stereoisomer D-arginine (3 mM) (data not shown).

3.3. Effects of suramin and Cibacron blue F3GA on IJPs evoked by intramural nerve stimulation

As shown in (Fig. 3), suramin (100–400 μM), a P2 receptor antagonist, exhibited concentration-dependent inhibition of IJP-F without changing the resting membrane potential ($n = 5$).

The type of the receptor mediating the IJP-F in response to nerve stimulation was assessed by application of CBF3GA (50–200 μM), a P2Y receptor antagonist. CBF3GA inhibited the IJP-F amplitude in a concentration dependent manner without affecting the resting membrane potential ($n = 5$) (Fig. 3).

3.4. Effect of P2Y receptor desensitization by 2-meSATP on IJP

For further confirmation of which subtype of P2 receptors mediated the effects of ATP, the putative P2Y receptor agonist 2-meSATP was used to desensitize P2Y receptor. Tissues were incubated for 30 min in 2-meSATP (50 μM) and the amplitude of the IJPs were recorded. Desensitization with 2-meSATP completely abolished the IJP-F either after single (from 17.3 ± 2.1 to 0.0 ± 0.0) or five-pulse stimuli (from 25.6 ± 3.2 to 0.0 ± 0.0) ($n = 4$) (Fig. 4).

3.5. Effect of P2Y1 receptor antagonist A3P5PS on EFS-evoked IJP

Application of the specific antagonist of P2Y1 receptor, A3P5PS, did not affect the resting membrane potential, but markedly inhibited ($P < 0.05$; $n = 4$) IJP-F without any effect on IJP-S when applied at 10 μM for 30 min (Fig. 5).

3.6. Effects of exogenous applications of ATP and NO

Exogenous application of ATP (10 μM–1 mM) produced concentration dependent hyperpolarization ($n = 5$, Fig. 6a). One

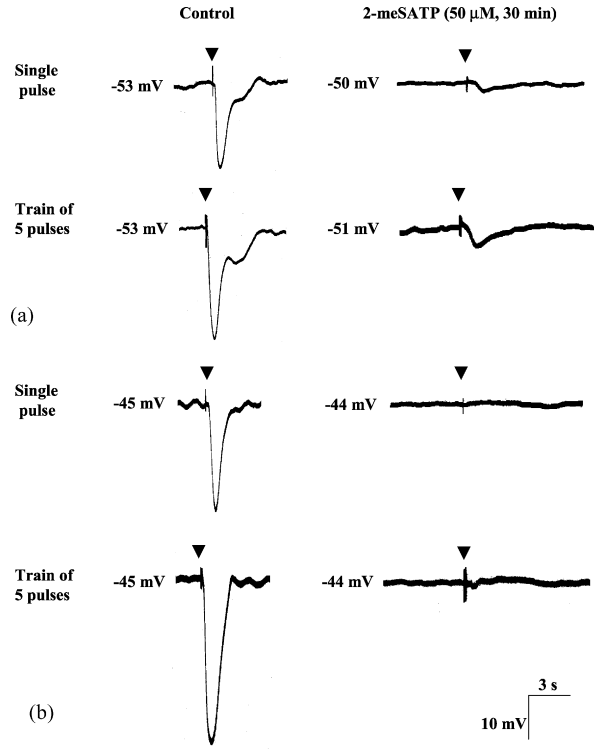

Fig. 4. Typical tracings showing the effect of desensitization of P2Y receptor putative agonist 2-methylthio-ATP (2-meSATP, 50 μM for 30 min) on junctional potentials evoked by EFS (0.5 ms, 50 V, single pulse and a train of five pulses at 25 Hz), applied at (▼) in the circular smooth muscle of hamster distal colon. 2-meSATP desensitization abolished IJP-F without any effects on IJP-S (a). Preincubation with L-NAME (200 μM) followed by 2-meSATP desensitization almost abolished both IJP-F and IJP-S (b).

micromolars ATP evoked a hyperpolarization of 20 ± 4.2 mV which occurred after a delay of 0.6 ± 0.1 s and time to peak of 2.2 ± 0.4 s. The membrane potential returned to its resting level after 35.8 ± 5.3 s. Similarly, exogenous application of NO solution (1–10 μM) evoked hyperpolarization but of slower onset, smaller amplitude and shorter duration. Ten micromolars of NO evoked a hyperpolarization of 9.8 ± 1.8 mV amplitude, 1.1 ± 0.3 s time to peak and 15 ± 2.6 s total duration to return back to the base line ($n = 5$) (Fig. 6b).

3.7. Effects of pertussis toxin and apamin on fast IJPs evoked by intramural nerve stimulation

P2Y receptors are variously coupled via PTX-sensitive and insensitive G-proteins to PLC, stimulating inositol triphosphate formation and Ca^{2+} release. For further identification of the P2Y receptor subtype through which ATP produces its IJP-F, the response was recorded after incubation of the colonic segment in 400 ng/ml PTX solution for 2 h. PTX significantly decreased the IJP-F amplitude being 33 ± 4.3 mV before and 11 ± 1.1 mV after PTX, respectively. Apamin effect on the IJP-F was also investigated because of the possible association of the PTX-sensitive P2Y receptor subtype with small conductance potassium channel. Apamin (0.3 μM) almost abolished the IJP-F response (Fig. 7a). Such experiments have been conducted in presence of L-NAME (200 μM).

3.8. Effects of ODQ and apamin on slow IJPs evoked by intramural nerve stimulation

In the hamster intestine, as NO was evidenced to produce its effect mainly via GC pathway that may end with opening of a small conductance K^+ channel, the IJP-S response was recorded after addition of the GC inhibitor, ODQ and apamin. ODQ (10 μM) or apamin (0.3 μM) significantly decreased the response (Fig. 7b).

3.9. Membrane response to strong EFS in presence of both purinergic and nitrergic blockers

In the presence of both purinergic and nitrergic blockers, EFS using trains of eight pulses or more at 25 Hz, evoked a slow hyperpolarization that was sensitive to the VIP antagonist, VIP 6–28 (1 μM). Furthermore, exogenous application of VIP (1–10 μM) produced similar response that was blocked also by the same specific antagonist (Fig. 8).

4. Discussion

Results of the present experiments provide evidence that ATP and NO released from colonic intramural nerves are the main NANC neurotransmitters responsible for evoking IJPs (fast and slow, respectively) in the circular muscle cells of hamster distal colon. An additional role of VIP is also indicated. The most substantial findings from which this conclusion has been derived are: (1) blockade of the IJP-F by universal and P2Y purinergic receptor blockers; (2) blockade of the IJP-S by nNOS and GC inhibitors; (3) blockade of strong EFS- and VIP-induced hyperpolarization in the presence of purinergic and nitrergic blockers by VIP 6–28, a VIP receptor antagonist.

ATP is a ligand for P2 purinoceptors existing in two main subtypes: (1) the P2X receptors that are ligand-gated ion channels and (2) the P2Y receptors that are coupled to G proteins [16]. Inhibitory responses are assumed to be mediated mainly through P2Y receptors. The main transduction pathway activated by binding of ATP to P2Y receptors involves the activation of phospholipase C, production of inisitol triphosphate and release of Ca^{2+} from intracellular stores [17]. This local Ca^{2+} transient activates Ca^{2+}-sensitive ion channels, such as SK_{Ca} channels leading to hyperpolarization and inhibition of voltage-operated Ca^{2+} channels with final relaxation of the supplied tissue [18]. Suramin [19] and CBF3GA [20] were described as universal P2 and selective P2Y receptor antagonists, respectively. Data of the present study showed that application of either of these drugs abolished IJP-F and ATP-evoked hyperpolarization indicating that the responsible mediator is ATP. The observation of inhibition of IJP-F and ATP effect with apamin suggests the involvement of SK channels in the hyperpolarizing response. Under our experimental conditions, desensitization of P2Y receptors by prolonged application of the putative P2Y agonist 2-meSATP abolished the IJP-F and blocked the effect of exogenous ATP giving further confirmation of the role of ATP.

More than one type of P2Y receptors have been cloned and functionally characterized, namely, P2Y1, P2Y2, P2Y4, P2Y6,

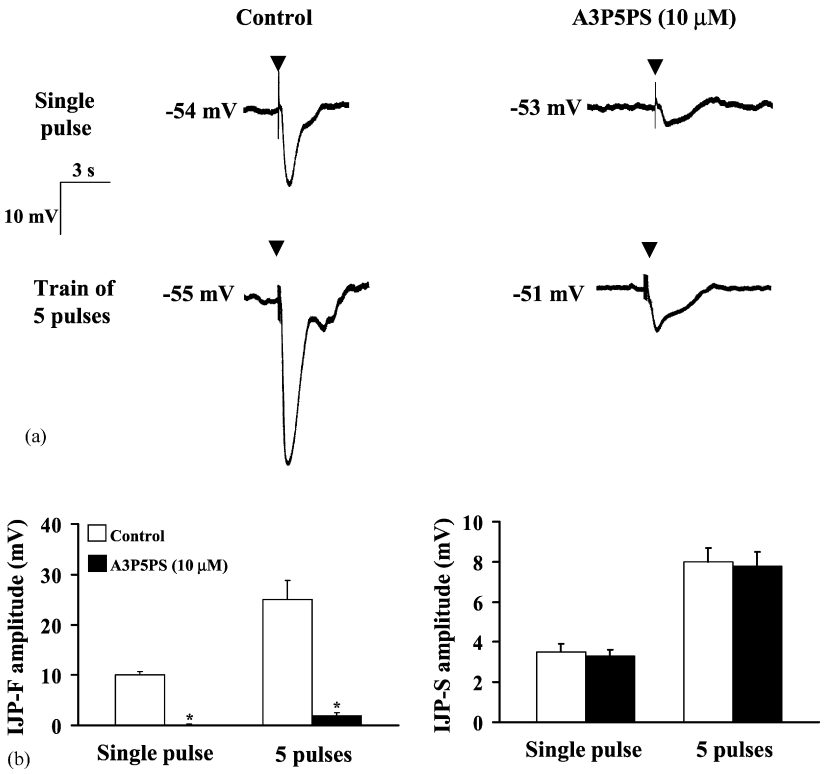

Fig. 5. Typical tracings (a) and histogram (b) showing the effect of the specific P2Y1 receptor antagonist, A3P5PS (10 μM) on junctional potentials evoked by EFS (0.5 ms, 50 V, single pulse and a train of five pulses at 20 Hz), applied at (▼) in the circular smooth muscle of hamster distal colon. A3P5PS almost abolished IJP-F without any effects on IJP-S. Each value is mean ± S.E. mean of four preparations. Significantly different from control values: $^{*}P < 0.05$.

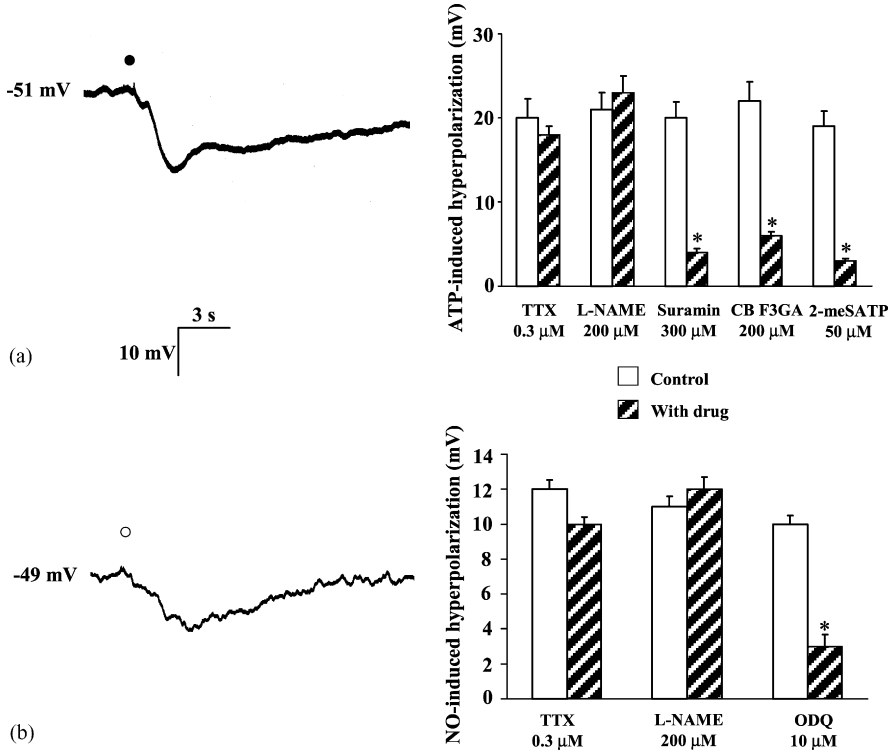

Fig. 6. Effects of exogenously applied ATP and NO on membrane potential of hamster distal colonic circular smooth muscle cells: (a) typical hyperpolarization evoked by exogenous ATP (1 mM), at (●) and summary effects of TTX (0.3 μM), L-NAME (200 μM), suramin (300 μM), CBF3GA (200 μM) and 2-meSATP desensitization (50 μM for 30 min) on it. (b) Typical hyperpolarization evoked by exogenous NO (10 μM), at (○) and summary effects of TTX, L-NAME and ODQ (10 μM) on it. Each value is mean ± S.E. mean of five preparations. Significantly different from control values: $^{*}P < 0.05$.

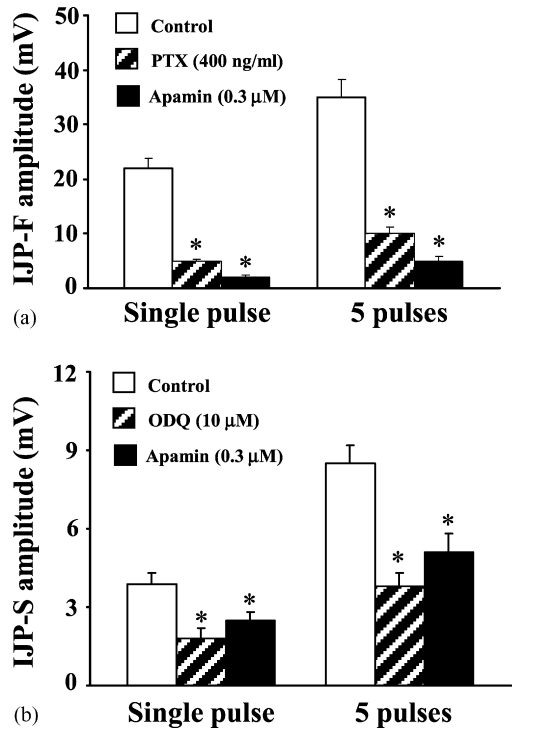

Fig. 7. Histograms showing: (a) the effects of G-protein inhibitor, pertussis toxin (PTX, 400 ng/ml for 2 h) and the small and intermediate Ca^{2+} sensitive K^+ channel blocker, apamin (0.3 μM) on IJP-F and (b) the effects of gyanylate cyclase inhibitor, 1H-[1,2,4]oxadiazolo-[4,3-a]quinoxalin-1-one (ODQ, 10 μM) and apamin on IJP-S; evoked by EFS (0.5 ms, 50 V, single pulse and a train of five pulses at 25 Hz) in the circular smooth muscle of hamster distal colon. Each value is mean ± S.E. mean of three preparations. Significantly different from control values: $^*P < 0.05$.

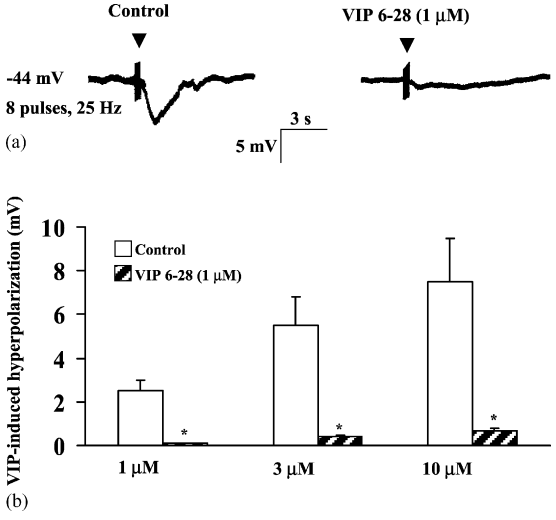

Fig. 8. Typical tracing and histograms showing the effects of the VIP antagonist, VIP 8-26 (1 μM) on IJP evoked by EFS (0.5 ms, 50 V, train of eight pulses at 25 Hz), delivered at (▼) (a) and exogenously applied VIP (1–10 μM; (b) in the circular smooth muscle of hamster distal colon in the presence of both purinergic and nitrergic blockers. Each value is mean ± S.E. mean of three preparations. Significantly different from control values: $^*P < 0.05$.

P2Y11, P2Y12, P2Y13 and P2Y14 [16]. These P2Y receptors are coupled to G-proteins that are sensitive or not to PTX. Responses mediated via G-proteins associated with P2Y1, P2Y6 and P2Y11 are not inhibited by PTX, where as those mediated via P2Y2 and P2Y4 receptors are partially sensitive to PTX [21]. Results presented in this study showed that EFS evoked IJP-F was partially inhibited by PTX indicating that this membrane response mediated via the action of ATP on P2Y2/P2Y4-like receptor subtype. However, our data also showed that the IJP-F was markedly inhibited by the P2Y1 receptor antagonist, A3P5PS [22]. This may be not consistent with the result of sensitivity of IJP-F to PTX as G-protein associated with P2Y1 receptor is PTX-non-sensitive [21]. Taken together, this could be explained on the basis of an interaction between P2Y1 and P2Y2 receptors on the circular smooth muscle cells of hamster distal colon; where, EFS-evoked ATP binds to P2Y1 receptor activating its associated G-protein (G_q) that consequently could activate the P2Y2-associated G-protein ($G_{i/o}$) which mediated the intracellular pathway producing the IJP-F response. Further studies are needed to explore the details of this interaction. Supportive evidence to this explanation is that receptor–receptor interaction has been previously reported among subtypes within a single group of receptors especially G-protein-coupled receptor family [23,24]. Moreover, in Ref. [16] authors have stated that P2Y1 and P2Y2 receptors are colocalized in some cells like those of some endothelia, but the biological significance of this colocalization is not clear.

The second component, IJP-S, has been found to be mediated via release of NO from nitrergic neurons and its diffusion into the circular muscle cell activating GC enzyme. This conclusion has been derived from the present results where TTX, neuronal blocker, L-NAME, NOS blocker, as well as ODQ, GC inhibitor, could inhibit the IJP-S. Although more than one report indicated that NO-mediated IJP is apamin-resistant [3,25], our data was inconsistent with that where apamin partially inhibited the IJP-S in the hamster distal colon. The result is consistent with [13] where NO-mediated response was apamin-sensitive in hamster proximal colon. The remaining component of IJP-S in the presence of either ODQ or apamin may indicate a direct effect of NO on ion channel rather than SK Ca^{2+}-sensitive K^+ ion channel. The later statement may be supported by finding of [26] that NO directly activates Ca^{2+}-dependent K^+ channels in smooth muscle of rabbit aorta.

It is worthy to mention that blocking of the IJP-S via L-NAME resulted in significant increase in IJP-F amplitude indicating an interaction between NO and ATP as coordinating inhibitory transmitters to circular muscle cells of hamster distal colon. It appears that NO decreases ATP release probably via a prejunctional mechanism. This data is consistent with what have been previously found in hamster proximal colon by our group [13]. It was also noticed that application of nitrergic blockers resulted in depolarization of the membrane potential with about 10 mV, but this was not the case after application of purinergic blockers or after purinergic receptor desensitization.

The third putative NANC inhibitory transmitter to hamster distal colon was VIP. Data of the present study showed that in presence of both purinergic and nitrergic blockers, EFS using

trains of eight or more pulses at 25 Hz results in small slow IJP that was sensitive to VIP receptor antagonist, VIP 6–28. This data may indicate a contributing role of VIP to the membrane inhibitory response. More than one study investigated the inhibitory roles of VIP and NO and the possible relation between them, which still a matter of debate. There are two theories have been raised to explain the relation between VIP and NO which could be briefly stated as: (1) VIP and NO act consequently or in a series, where VIP released by enteric nerve firing stimulates NO synthesis in both neuronal nerve endings and smooth muscle cells resulting in an NO-mediated relaxation; (2) VIP and NO act independently or in parallel through completely distinct mechanisms producing inhibition via adenylate cyclase (AC) and GC, respectively [27]. A third theory has been added by our group [28] where the released NO mediates its inhibitory effect both directly through its muscular action and indirectly via enhancing the release of VIP through a prejunctional action on VIP-containing neurons. Evoking a hyperpolarizing response after strong EFS in presence of the NOS blocker, L-NAME, may refer to the validity of the second theory (parallel pathways) in hamster distal colon, however, the other theories could not be also excluded indicated by absence of the response after application of weaker EFS.

The present data extended the evidence of presence of species and regional variation in the neurotransmitters mediating a membrane response. It is consistent with those reported in guinea pig colon [3], partially consistent with those reported in mouse distal colon [12], but inconsistent with those recorded from rat colons [4].

Data of the present study provides supportive evidence for the contributing roles of ATP, NO and VIP as NANC inhibitory neurotransmitters to the circular muscle cells of the hamster distal colon. ATP is responsible for the fast IJP while NO and VIP are responsible for the slow IJP. ATP-mediated response is dependent on P2Y1/P2Y2 receptors while NO and VIP responses are dependent on GC and AC enzyme systems, respectively. In addition, the results may indicate an interactions between NO and ATP, however, a relationship between NO and VIP could not be accurately determined.

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