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Abstract

Effects of mesenteric nerve (MN) stimulation on the electrophysiological behavior of myenteric neurons in the guinea pig ileum were investigated with intracellular recording techniques in the myenteric flaps innervated with mesenteric nerves. MN stimulation at 0.11-6 Hz evoked fast excitatory postsynaptic potentials (EPSPs) in 6 myenteric neurons (2 Type 2/AH, 3 NS and 1 Type 1/S cells) and rarely evoked antidromic soma spike potentials in 3 myenteric neurons. Fast EPSPs were abolished by hexamethonium. Slow EPSPs evoked by MN stimulation (Takaki and Nakayama (1988) *Brain Res.*, 442, 351-353) were also obtained in 5 Type 2/AH neurons and were irreversibly abolished by superfusion with capsaicin 10 microM. It is, therefore, likely that fast EPSPs mediated by nicotinic cholinergic receptors are due to stimulation of the vagus nerve and slow EPSPs are mediated by a release of substance P at axosomatic synapses due to antidromic activation of the capsaicin-sensitive sensory nerves.

KEYWORDS: autonomic ganglion, intestine, mesenteric nerve (MN) stimulation, myenteric neuron

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Electrical Behavior of Myenteric Neurons Induced by Mesenteric Nerve Stimulation in the Guinea Pig Ileum

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Effects of mesenteric nerve (MN) stimulation on the electrophysiological behavior of myenteric neurons in the guinea pig ileum were investigated with intracellular recording techniques in the myenteric flaps innervated with mesenteric nerves. MN stimulation at 0.11-6 Hz evoked fast excitatory postsynaptic potentials (EPSPs) in 6 myenteric neurons (2 Type 2/AH, 3 NS and 1 Type 1/S cells) and rarely evoked antidromic soma spike potentials in 3 myenteric neurons. Fast EPSPs were abolished by hexamethonium. Slow EPSPs evoked by MN stimulation (Takaki and Nakayama (1988) *Brain Res.*, 442, 351-353) were also obtained in 5 Type 2/AH neurons and were irreversibly abolished by superfusion with capsaicin $10\mu\text{M}$. It is, therefore, likely that fast EPSPs mediated by nicotinic cholinergic receptors are due to stimulation of the vagus nerve and slow EPSPs are mediated by a release of substance P at axosomatic synapses due to antidromic activation of the capsaicin-sensitive sensory nerves.

Key words : autonomic ganglion, intestine, mesenteric nerve (MN) stimulation, myenteric neuron

It has been reported that cholinergic contraction of guanethidine-treated isolated guinea-pig ileum to mesenteric nerve (MN) stimulation involves hexamethonium-sensitive and capsaicin-sensitive components (1). On the other hand, synaptic behavior of enteric neurons evoked by stimulation of the fiber tract or the ganglion of the myenteric plexus has been well described for the guinea pig small intestine (2-4). Fewer reports are available on synaptic behavior of intrinsic ganglion cells receiving input from only extrinsic nerves (5, 6). An intracellular study of myenteric neurons of guinea pig small intestine (5) indicated that MN stimulation evoked the inhibitory effect

on the excitatory postsynaptic potentials (EPSPs) by transmural stimulation and rarely evoked a persistent discharge of EPSPs and that such EPSPs are presumably mediated by the release of acetylcholine (Ach). Recently, we have reported that MN stimulation (at 20 Hz) evoked slow EPSPs, which mimic the slow depolarizing action induced by exogenous substance P, in myenteric neurons on the myenteric flaps innervated with mesenteric nerves in the guinea pig isolated ileum (6). We have now examined more extensively the effects of MN stimulation on the electrical behavior of myenteric neurons.

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Materials and Methods

Segments of intestine were removed from the ileum of adult guinea pigs that had been stunned by a blow to the head and exsanguinated. Flat sheet preparations of longitudinal muscle with the myenteric plexus attached were made, which were innervated with mesenteric nerves from the middle parts of the preparations bi-directionally to both oral and anal ends (1). The tissues were mounted in a superfusion chamber and viewed with a binocular stereomicroscope, lighted by a microelectrode illumination system (Narishige, Tokyo, Japan). Glass micropipettes filled with 3 M KCl were used for recording and had resistance of 40–80 M Ω . The high-impedance preamplifier (Nihon-Kohden, Tokyo, Japan) containing bridge circuitry was used for recording membrane and action potentials, and injecting electrical current into myenteric neurons. Some of the records were played back on a Reticorder (Nihon-Kohden, Tokyo, Japan) from data previously recorded on magnetic tape. Electrical stimulation of mesenteric nerves (0.11–20 Hz, 0.5–1 ms, supramaximal currents) was given through a pair of annular platinum electrodes. The tissues were maintained in Krebs solution (6–9) containing guanethidine 2 μ M to avoid any influence of adrenergic nerves involved in mesenteric nerves at 36°C and gassed with 95% O₂–5% CO₂. The tissue chamber was perfused at a rate of 11–12 ml/min. The perfusate completely reached the tissue chamber 3–4 min after onset of the perfusion. Drugs used were capsaicin (Sigma Chemical Company, St. Louis, MO, USA), guanethidine sulfate (Tokyo Kasei Kogyo, Tokyo, Japan) and hexamethonium bromide (Sigma Chemical Company, St. Louis, MO, USA). Capsaicin (10 mM) was dissolved in 100% dimethylsulfoxide (DMSO). When used, the stock solution was diluted one-thousand-fold with Krebs solution. The final concentration of DMSO was 0.1%. DMSO at 1% had no effect of its own.

Results

Effects of MN stimulation were examined on 51 myenteric neurons from 24 guinea pigs. These neurons were classified into 3 categories using criteria that had been previously described (3, 4, 6–10). Type 1/S cells were defined as those with a high input resistance, which spiked repeatedly during the injection of a depolarizing

current and displayed anodal-break excitation. Type 2/AH cells were defined as those with a low input resistance, which displayed a character-

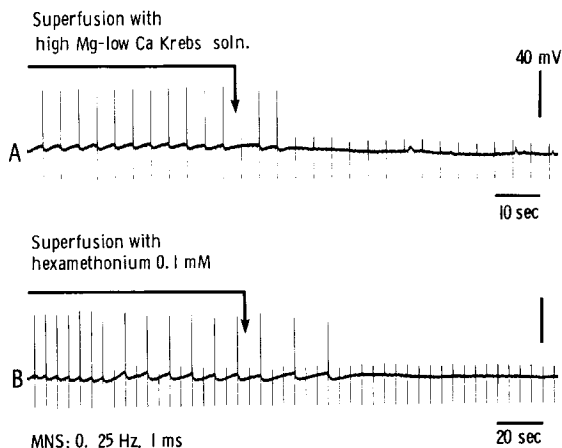


Fig. 1 Fast excitatory postsynaptic potentials (EPSPs) with action potentials induced by mesenteric nerve stimulation (MNS : 0.25 Hz, 1 ms) in a Type 2/AH neuron. At the arrow, the superfusate has completely entered the tissue chamber. In (A), superfusion with high magnesium (12.5 mM) and low calcium (1.25 mM) Krebs solution and in (B), superfusion with hexamethonium 0.1 mM blocked the fast EPSPs with action potentials. Each downward deflection was an artifact of stimulus. The resting membrane potential was -65 mV.

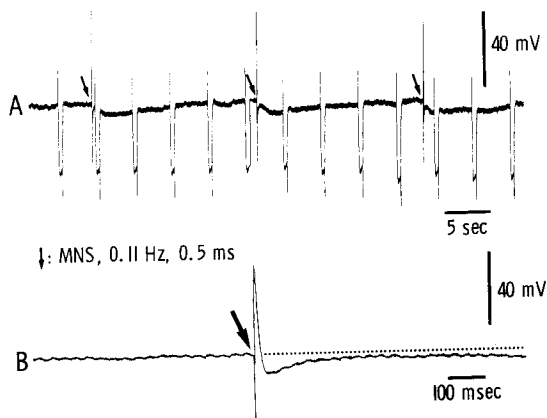


Fig. 2 Antidromic spike potentials evoked by mesenteric nerve stimulation (MNS : 0.11 Hz, 0.5 ms ; at the arrow) in a Type 2/AH neuron. In (A), hyperpolarizing current pulses were injected to monitor the changes of input resistance. In (B), a trace of recording by faster speed is shown. At arrow, stimulus was delivered and caused a narrow spike potential followed by a long-lasting afterhyperpolarization. The resting membrane potential was -58 mV.

istic prolonged afterhyperpolarization (AH) following an action potential. NS cells were defined as those cells that did not spike in response to injection of depolarizing current or application of drugs. The sample studied included 18 Type 2/AH cells, 30 NS cells and a rather small number ($n=3$) of Type 1/S cells. In 6 myenteric neurons (2 Type 2/AH, 3 NS and 1 Type 1/S cells), MN stimulation at frequency (0.11–6 Hz) evoked fast EPSPs or fast EPSPs with action potentials (Fig. 1). These fast EPSPs in some responsive neurons tested increased in amplitude when the membrane was hyperpolarized by intrasomal current injection and decreased in amplitude when the membrane was depolarized. The amplitude of fast EPSPs in myenteric neurons of the guinea pig small intestine becomes progressively smaller when they are evoked repeatedly by electrical stimulation of the fiber tracts or ganglionic surface (11). The rate of this run-down is a direct function of stimulus frequency. In the present study, at 10 Hz a marked run-down was observed. However, no run-down was observed at stimulus frequency as low as 0.25 Hz (Fig. 1). Superfusion of the tissue with a nicotinic antagonist, hexamethonium (0.1 mM) abolished fast EPSPs with action potentials as shown in Fig. 1, B. Superfusion with high magnesium (12.5 mM) and low calcium (1.25 mM) Krebs solution also abolished these fast EPSPs as shown in Fig. 1, A.

MN stimulation at low frequency (0.11 Hz) evoked antidromic spike potentials in only 3 myenteric neurons (2 Type 2/AH and 1 NS cells). The amplitude of the spikes was unchanged when the membrane potential was current clamped to levels more positive or negative than the resting potential, and the antidromic spike potentials were unaffected by hexamethonium or by high magnesium and low calcium media. The duration of the antidromic spike potentials was significantly shorter than fast EPSPs (Fig. 2, B)(cf. Fast synaptic events are less than 80 ms in duration)(3).

MN stimulation at frequency 20 Hz for 20–40

sec evoked slow EPSPs, which have been characterized previously (6), in 5 Type 2/AH neurons in the present study. These slow EPSPs mimic the slow depolarizing responses to exogenous substance P (6). As shown in Fig. 3, A, MN stimulation produced a long-lasting slow EPSP associated with an increased input resistance (157.7% of the control) in a Type 2/AH neuron. Superfusion with capsaicin $10\mu\text{M}$ provoked a long-lasting slow depolarization associated with an increased input resistance, as described previously (9), which mimics the slow EPSP induced by MN stimulation. At the crest of the slow depolarization, anodal-break excitation (full soma spike potential) at the termination of hyperpolarizing current pulses which indicates augmented excitability of a cell was observed. Four min after

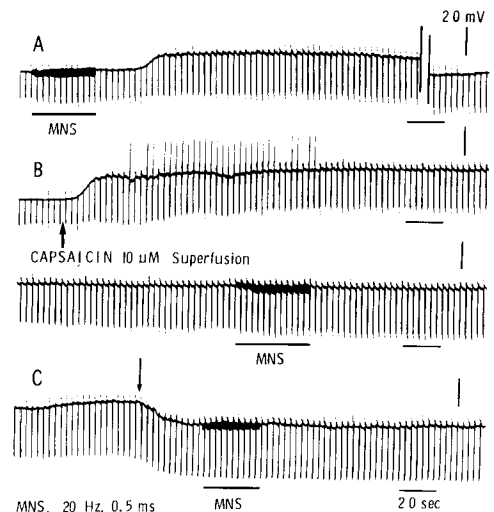


Fig. 3 Effect of superfusion with capsaicin $10\mu\text{M}$ on the slow excitatory postsynaptic potentials (EPSPs) induced by mesenteric nerve (MN) stimulation (MNS : 20 Hz, 0.5 ms) in a Type 2/AH neuron. A, control response ; MN stimulation caused a long-lasting slow depolarizing response associated with an increased input resistance. After 6 min the membrane potential recovered to the resting level. B, capsaicin per se caused a slow depolarizing action, which is similar to the slow EPSP by MN stimulation. Four min after superfusion with capsaicin, the slow EPSP by MN stimulation was abolished. C, twenty-four min after capsaicin, at the arrow, membrane potential was hyperpolarized to the resting level, but MN stimulation did not cause any effect. Sixty-nine min after thorough washing, the response to MN stimulation did not recover. The resting membrane potential was -68mv .

superfusion with capsaicin the response to MN stimulation was abolished (Fig. 3, B). Even 69 min after thorough washing, the response did not recover.

No neurons which indicate both slow EPSPs and fast EPSPs (or antidromic spikes) could be obtained in the sample currently studied. Therefore, no response to MN stimulation was observed in 37 (9 Type 2/AH, 2 Type 1/S and 26 NS cells) out of 51 neurons.

Discussion

Synaptic behavior of myenteric neurons evoked by stimulation of the fiber tract and the ganglion of the myenteric plexus (2-4) has been well described. Slow synaptic modulation of excitability within the myenteric plexus is known to involve a reduction of both Ca^{2+} -associated resting potassium conductance (g_K) and post-spike g_K which is secondary to suppression of Ca^{2+} -influx by the neurotransmitter, such as substance P for the slow EPSP (12-15). Therefore, the slow EPSPs by MN stimulation may possibly be generated by the similar ionic mechanism.

It has been reported that the slow EPSP induced by orthodromic electrical stimulation of afferent fibers in the ureter nerve (16) and lumbar colonic nerve (17) in the guinea pig inferior mesenteric ganglion neurons have been attenuated or abolished by capsaicin. Antidromic stimulation of capsaicin-sensitive sensory afferent fibers of substance P-containing neurons, soma of which are located in the spinal dorsal root ganglia, could evoke a release of substance P from the sensory nerve terminal into the myenteric plexus (1, 6). Present results indicate that slow EPSPs by MN stimulation are due to antidromic activation of capsaicin-sensitive sensory nerves involved in mesenteric nerves and are presumably mediated by the release of sensory neuropeptides, such as substance P. Long-lasting blockage of slow EPSP by capsaicin is thought to be due to a

depletion of a releasable pool of sensory neuropeptides, although the possibility of other mechanisms can not be excluded. Therefore, it seems likely that these responses of myenteric neurons contribute to the capsaicin-sensitive cholinergic contractile response to MN stimulation at 20 Hz in the guanethidine-treated isolated ileum (1). Fast EPSPs by MN stimulation were also obtained in the present study. This result indicates that there exists a nicotinic cholinergic synaptic input from mesenteric nerves to myenteric neurons. The nerve fibers that cause this synaptic event may be involved in the vagus nerve, since the mesenteric nerve contains a small proportion of vagal parasympathetic fibers (5). However, it is unknown whether this nicotinic transmission may contribute to the hexamethonium-sensitive cholinergic contractions induced by MN stimulation in the guanethidine-treated guinea pig isolated ileum (1), since the 'run-down' phenomenon of fast EPSPs must occur when the mesenteric nerve is stimulated at frequency 20 Hz.

Antidromic spike potentials evoked by MN stimulation in the present study may be due to antidromic activation of a nerve fiber in the mesenteric nerves from the cell body of the myenteric neuron projecting into the prevertebral ganglion (18). It is unknown whether under physiological conditions these fibers behave like axons and conduct spike information away from the cell body or whether they behave like dendrites and normally conduct toward the cell body under functional conditions *in situ*.

About 70 % of neurons currently studied did not respond to MN stimulation. This may reflect a scarce, direct innervation with mesenteric nerves (non-adrenergic) to myenteric neurons.

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