Cholinergic Modulation and Effects of Dynorphin on the Non-adrenergic Inhibitory Potentials in the Guinea-pig Duodenum

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Abstract  Cholinergic modulation and effects of dynorphin on the non-adrenergic non-cholinergic inhibitory potentials (NANC i.p.s) in the longitudinal smooth muscle cells of the guinea-pig duodenum were studied intracellularly. Atropine (1.4 x 10^{-7} - 1.4 x 10^{-5} M) and scopolamine (3.3 x 10^{-8} - 3.3 x 10^{-7} M) increased the amplitude of the evoked i.p.s while physostigmine (3.7 x 10^{-7} - 3.7 x 10^{-6} M) and neostigmine (4.8 x 10^{-8} M) decreased it. The frequency of the spontaneous action potentials in the longitudinal smooth muscle cells was increased by dynorphin (6 x 10^{-8} - 3 x 10^{-7} M) without continuous membrane depolarization. The excitatory effect of dynorphin on the spontaneous electrical activity was not blocked by atropine (1.4 x 10^{-6} M). Dynorphin (6 x 10^{-8} - 2.4 x 10^{-7} M) increased the amplitude of the i.p.s evoked in the presence of atropine (1.4 x 10^{-7} - 1.4 x 10^{-6} M) and in the propranolol (3.9 x 10^{-6} M) solution containing atropine while dynorphin decreased the amplitude of the i.p.s evoked in the absence of atropine. In the high calcium solution containing atropine, the amplitude of the i.p.s increased. Further increase in the amplitude of the i.p.s was observed by additively applied dynorphin. However, the amplitude of the i.p.s evoked in the high calcium solution without atropine was decreased by dynorphin. These results suggest the cholinergic inhibitory modulation on the NANC inhibitory nerves in the guinea-pig duodenum, the non-cholinergic excitatory action of dynorphin on the spontaneous electrical activity of the longitudinal smooth muscle and the excitatory action of dynorphin on the NANC inhibitory nerves in the presence of muscarinic blocking agents.

Key words: non-adrenergic inhibitory potential, cholinergic modulation, dynorphin, duodenal smooth muscle.

In the mammalian gastrointestinal tract, intestinal smooth muscles are innervated by two different nervous systems. These are cholinergic excitatory and inhibitory systems. The latter includes adrenergic and non-adrenergic non-
cholinergic (NANC) systems. The NANC inhibitory system has also been shown in cat and guinea-pig airways (Coburn and Tomita, 1973; Diamond and O'Donnell, 1980; Altieri et al., 1985). Recently, it was reported that the NANC inhibitory system function in cat airways is neither dependent upon nor modulated by neurally released acetylcholine (Altieri et al., 1985). The role of acetylcholine in modulating the NANC i.p.s in the intestinal tract has not been investigated. The aim of the present experiments is to make clear the cholinergic modulation on the NANC inhibitory system in the intestinal tract.

Recent investigations revealed that immunocytochemical staining of guinea-pig ileum showed a heavy concentration of dynorphin-positive fibers in the submucous plexus (Watson et al., 1981) and that dynorphin, an endogenous opioid peptide, was present in porcine duodenum (Tachibana et al., 1982). It was shown that the release of dynorphin from the guinea-pig small intestine related to peristalsis (Kromer et al., 1981; Donnerer et al., 1984). Oka et al. (1982) have demonstrated that dynorphin acts as an endogenous agonist on \( \kappa \)-opiate receptors in guinea-pig and rabbit ileum. These results suggest that dynorphin regulates the intestinal motility. However, the role of dynorphin in the neural control of the intestinal smooth muscles is not known. The present experiments were also carried out to investigate the role of dynorphin on the NANC inhibitory neurotransmission in the guinea-pig duodenum. Some of the results have been communicated (Ohkawa, 1985).

METHODS

Guinea-pigs of either sex weighing 300–400 g were stunned and bled, and a short segment (about 3 cm from the pylorus of the stomach) of the duodenum was removed. The duodenum was opened along the mesenteric border and full-thickness strips (4 mm x 2 mm) were cut parallel to the longitudinal direction. These strips were pinned on a rubber plate in an organ bath of 2 ml volume superfused with warmed Krebs solution (36°C) at a rate of 4 ml/min. The membrane activity of the longitudinal smooth muscle cells of the preparation was recorded with intracellular micro-electrodes placed less than 1 mm from one of the stimulating electrodes, separated by 4 mm, in response to rectangular pulses (usually 0.3 ms duration at constant strength).

The modified Krebs solution contained (mm): NaCl 122, KCl 4.9, NaHCO\(_3\) 15.5, KH\(_2\)PO\(_4\) 1.2, CaCl\(_2\) 2.5, MgCl\(_2\) 1.2, and glucose 11.5. The drugs used are as follows: atropine sulfate, dynorphin-(1-13) (Peptide Institute Inc.), guanethidine hydrochloride, hexamethonium bromide (Nakarai Chemicals), neostigmine bromide (Sigma), phentolamine hydrochloride (Regitine, CIBA), physostigmine salicylate (Merck), propranolol hydrochloride (Inderal, Sumitomo), scopolamine hydrobromide (Wako), \( \alpha \)-tubocurarine (Tokyo Kasei), and tetrodotoxin (Sigma). Values of the measured parameters of muscle membrane and inhibitory potentials were expressed as the mean ± S.D. (\( n = \) number of penetrations of the micro-
The mean resting membrane potential of the longitudinal smooth muscle cells of the guinea-pig duodenum was $-51.4 \pm 2.9$ mV ($n=36$). Field stimulation elicited an inhibitory potential (i.p.) in the longitudinal smooth muscle. The i.p.s were

![Fig. 1. Effects of atropine (A) and scopolamine (B) on the evoked i.p.s in the longitudinal smooth muscles (right panels; fast sweep recordings). A and B were obtained from different preparations.](image)

RESULTS

The mean resting membrane potential of the longitudinal smooth muscle cells of the guinea-pig duodenum was $-51.4 \pm 2.9$ mV ($n=36$). Field stimulation elicited an inhibitory potential (i.p.) in the longitudinal smooth muscle. The i.p.s were...
observed in all cells, the amplitude varying up to 28 mV. The i.p.s were not blocked by atropine (1.4 × 10⁻⁶ M), guanethidine (3.4 × 10⁻⁶ M), phentolamine (3.6 × 10⁻⁶ M), propranolol (3.9 × 10⁻⁷ M), and hexamethonium (5 × 10⁻⁶ M). Tetrodotoxin (3.1 × 10⁻⁷–3.1 × 10⁻⁶ M) blocked the evoked i.p.s. In the present experiments, spontaneous i.p.s and excitatory junction potentials (e.j.p.s) and evoked e.j.p.s in the longitudinal smooth muscle cells were not detected. The drug effects on the i.p.s were observed after 5–10 min of perfusion.

Fig. 2. Effects of physostigmine (A and B) and neostigmine (C) on the i.p.s. In C, a repeated stimulation (4 Hz, 20 pulses) was applied. A and C were obtained from different preparations.
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Fig. 3. Changes in the relative amplitude of the i.p.s evoked in atropine (A), scopolamine (B), and physostigmine (C) at various concentrations. **p<0.01.

Relative amplitude of i.p.
Effects of atropine, scopolamine, and physostigmine on the inhibitory potentials

The i.p.s were evoked by trains of 10 to 20 pulses at a frequency of 0.5 Hz. Atropine (1.4 x 10^-7 to 1.4 x 10^-5 M) increased the amplitude of the i.p.s. In a series of experiments, the mean amplitudes of the i.p.s were 18.3 ± 2.1 mV (n = 78) in normal solution and 22.3 ± 0.9 mV (n = 46, p < 0.001) at 1.4 x 10^-6 M atropine. The prolongation of the time to peak and the half duration of the i.p. was observed. The latency of the i.p. was not changed. Figure 1A shows the i.p.s evoked in normal and atropine solutions. The amplitude of the i.p.s was not affected by the treatment with guanethidine (5 x 10^-6 M) and propranolol (3.9 x 10^-7 M) but it was significantly increased by additional application of atropine (1.4 x 10^-7 M; 147% of control in normal solution, n = 38).

Scopolamine (3.3 x 10^-8 to 3.3 x 10^-7 M) also increased the amplitude of the evoked i.p.s (Fig. 1B). The mean amplitudes of the i.p.s were 8.8 ± 1.3 mV (n = 52) in normal solution, 13.0 ± 0.7 mV (n = 31, p < 0.001) at 3.3 x 10^-8 M and 12.2 ± 1.1 mV (n = 45, p < 0.001) at 3.3 x 10^-7 M scopolamine. Similar changes in the time course of the i.p. to these in atropine solution were detected. The resting membrane potential of the longitudinal smooth muscle cells was not changed in these solutions.

The amplitude of the i.p.s was decreased by physostigmine (3.7 x 10^-7 to 3.7 x 10^-6 M). The inhibitory effect of physostigmine on the i.p. was shown in Fig. 2A and

Fig. 4. Effects of dynorphin on the spontaneous action potentials in the longitudinal smooth muscle of the duodenum. A, control. B, dynorphin (DY) 6 x 10^-8 M. C, 10^-7 M. D, 3 x 10^-7 M. E, recovery. Records in B-D were obtained after 5-6 min in dynorphin.

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B. The mean amplitudes of the i.p.s were $24.4 \pm 2.0 \text{mV} \,(n=28)$ in normal solution and $17.1 \pm 2.6 \text{mV} \,(n=52, \,p<0.001)$ at $3.7 \times 10^{-6} \text{M}$ physostigmine. Neostigmine ($4.8 \times 10^{-8} \text{M}$) had a similar inhibitory effect on the i.p.s to that of physostigmine. Figure 2C shows the inhibitory effect of neostigmine on the i.p.s evoked by repeated stimulation (4 Hz, 20 pulses). Treatment with $d$-tubocurarine ($1.4 \times 10^{-7} \text{M}$) did not increase the amplitude of the i.p.

The excitatory and inhibitory effects of atropine, scopolamine, and physostigmine on the relative amplitude of the i.p.s were summarized in Fig. 3. The relative amplitude of the i.p. was dose-dependently increased by atropine and decreased by physostigmine.

Effects of dynorphin on the spontaneous action potentials in the longitudinal smooth muscle

The resting membrane potential of the longitudinal smooth muscle cells was not changed by dynorphin ($0.3 \times 10^{-8} \text{--} 3 \times 10^{-7} \text{M}; \, -49.5 \pm 2.7 \text{mV} \,(n=15)$ in dynorphin $3 \times 10^{-7} \text{M}$; no statistical significance). Figure 4 shows the effects of dynorphin ($6 \times 10^{-8} \text{--} 3 \times 10^{-7} \text{M}$) on the spontaneous action potentials in the longitudinal smooth muscle cells. In normal solution, the burst type of spontaneous spike activity was observed. Dynorphin ($6 \times 10^{-8} \text{M}$) increased the frequency of the spontaneous action potential and produced the continuous spike activity (Fig. 4). The mean frequencies of the spontaneous action potential were $6.3/10\text{s}$ at $6 \times 10^{-8} \text{M}$ and $13.3/10\text{s}$ at $3 \times 10^{-7} \text{M}$ dynorphin. The increase in the frequency of spontaneous action potential seems to depend on the external dynorphin concentrations (Fig. 4B–D). The excitatory effect of dynorphin was reversible (Fig. 4E).

The spontaneous spike activity of the longitudinal smooth muscles was slightly suppressed by atropine ($1.4 \times 10^{-6} \text{M}$); the duration of the spike burst was shortened (Fig. 5A). In this solution, dynorphin ($6 \times 10^{-8} \text{M}$) increased the frequency of the

![A](image1.png)

**Atropine 1.4x10^8 M**

![B](image2.png)

**+ DY 6x10^7 M**

**Fig. 5.** Effects of dynorphin on the spontaneous action potentials in the longitudinal smooth muscle in the presence of atropine. A, atropine $1.4 \times 10^{-6} \text{M}$. B, dynorphin (DY) $6 \times 10^{-8} \text{M}$ in the presence of atropine.
spontaneous action potential (Fig. 5B). The mean frequency of the spontaneous action potential was 8.4/10 s in dynorphin containing atropine solution. Thus, the excitatory action of dynorphin was not blocked by atropine.

**Effects of dynorphin on the inhibitory potentials with and without atropine**

Dynorphin $3 \times 10^{-8}$ M had nearly no effect on the elicited i.p.s but the amplitude of the i.p.s decreased with increasing the concentration of dynorphin. The mean values of the amplitude of i.p.s were $23.8 \pm 1.8$ mV ($n = 80$) in normal solution and $15.8 \pm 1.3$ mV ($n = 90; p < 0.001$) in dynorphin $2.4 \times 10^{-7}$ M. The effects of dynorphin ($3 \times 10^{-8} - 2.4 \times 10^{-7}$ M) on the evoked i.p.s are shown in Fig. 6B–D and summarized in Fig. 6E.

In the presence of atropine ($1.4 \times 10^{-7} - 1.4 \times 10^{-6}$ M), dynorphin ($6 \times 10^{-8} - 2.4 \times 10^{-7}$ M) increased the amplitude of the i.p.s (Fig. 7A). Changes in the relative amplitude of the i.p.s by dynorphin are shown in Fig. 7B. When dynorphin $1.2 \times 10^{-7}$ M was applied additively, the mean amplitude of the i.p.s was 241% of control in normal solution ($n = 54, p < 0.001$). From the present results, the increase in the amplitude of i.p.s seems to depend on atropine and dynorphin concentrations. When a repeated stimulation (2 Hz, 10 pulses) was applied in dynorphin containing atropine solution, the amplitude of the i.p.s was increased and the post-stimulus depolarization was potentiated (Fig. 7A). In the presence of $d$-tubocurarine ($1.4 \times$
10^{-7} \text{ M}), dynorphin (6 \times 10^{-8} \text{ M}) did not increase the amplitude of evoked i.p.s but decreased it.

The i.p.s were not blocked by phentolamine and propranolol. Dynorphin (6 \times 10^{-8} \text{ M}) increased the amplitude of the i.p.s elicited in the atropine (1.4 \times 10^{-7} \text{ M}) containing propranolol (3.9 \times 10^{-7} \text{ M}) solution. The mean value of the increased amplitude of the i.p.s by dynorphin was 197% of control (n=45, p<0.001) in the atropine containing propranolol solution. However, in the presence of propranolol (3.9 \times 10^{-6} \text{ M}) or phentolamine (3.6 \times 10^{-7} \text{ M}) without atropine, additively applied dynorphin (3-6 \times 10^{-8} \text{ M}) decreased the amplitude of the i.p.s.

**Effects of dynorphin on the inhibitory potentials in various concentrations of calcium with and without atropine**

It was known that the amplitude of the evoked i.p.s in the intestinal smooth muscle cells was dependent on the external calcium concentrations (HOLMAN and WEINRICH, 1975; OHKAWA, 1984). In the following experiments, the effects of dynorphin on the i.p.s were examined in the high and low calcium concentrations with and without atropine.
When the external calcium concentration increased up to 5 mM, the amplitude of the i.p.s was increased (132% of control, \( n = 66, \ p < 0.001 \)). In this state, dynorphin (\( 6 \times 10^{-8} \) M) reduced the amplitude of the i.p.s (66% of control in the normal solution, \( n = 30, \ p < 0.001 \)). On the contrary, the mean amplitude of the i.p.s was reduced by the low calcium solution (1.25 mM \( \text{Ca}^{2+} \), 76% of control, \( n = 36, \ p < 0.001 \)). Further decrease in the amplitude of the i.p.s was detected by additively applied dynorphin (\( 6 \times 10^{-8} \) M, 70% of control in the normal solution, \( n = 81, \ p < 0.001 \)). Figure 8 summarized the effects of dynorphin and external calcium concentrations on the relative amplitude of the i.p.s.

Figure 9A shows the effects of dynorphin on the i.p.s in the presence of high calcium (5 mM) and atropine. The amplitude of the i.p.s evoked in the presence of atropine (1.4 \( \times 10^{-7} \) M) and high calcium was 117% of control in normal solution (\( n = 46, \ p < 0.001 \)). Additively applied dynorphin (\( 6 \times 10^{-8} - 1.2 \times 10^{-7} \) M) further increased the amplitude of the i.p.s, that is, the mean values of the amplitude of the i.p.s were 139% of control in normal solution (\( n = 38 \) at \( 6 \times 10^{-8} \) M, and 148% of control in normal solution (\( n = 33 \) at \( 1.2 \times 10^{-7} \) M dynorphin, respectively (Fig. 9C).

The effects of dynorphin on the i.p.s were examined in the low calcium solution including atropine. The increase in amplitude of the i.p.s by additively applied dynorphin (\( 6 \times 10^{-8} \) M) was not detected in this solution.

**DISCUSSION**

In the longitudinal smooth muscles of the guinea-pig duodenum, the amplitude
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of the evoked i.p.s was increased by atropine and scopolamine and decreased by physostigmine and neostigmine. These results suggest that the presence of the cholinergic inhibition on the NANC inhibitory nerves in duodenal wall and that the cholinergic receptors in the NANC inhibitory nerves are muscarinic. The NANC inhibitory system was found in the airways of the cat (Diamand and O'Donnell, 1980; Altiere et al., 1985) and the guinea-pig (Coburn and Tomita, 1973) and the suggestion was made that the NANC inhibitory system in cat airways is not modulated by the cholinergic system (Altiere et al., 1985). As, in the smooth muscle cells of cat trachea in which the NANC i.p.s were not detected, atropine did not alter the membrane property (Ito and Takeda, 1982), the NANC inhibitory system in the alimentary canal appears to differ from that in the cat airway.

Dynorphin increased the amplitude of the i.p.s in the longitudinal smooth muscles evoked in the presence of atropine. The preincubation with atropine brings

Fig. 9. Effects of dynorphin on the i.p.s evoked in the high calcium solution including atropine. A1, control i.p.s evoked in the high calcium (5 mM) and atropine $(1.4 \times 10^{-7} M)$. A2, i.p.s evoked in the high calcium, atropine with dynorphin (DY) $(6 \times 10^{-8} M)$. A3, i.p.s evoked in the high calcium, atropine with dynorphin $(1.2 \times 10^{-7} M)$. The i.p.s recorded by two different sweeps are shown in A. B, i.p.s evoked by a repeated stimulation (2 Hz, 10 pulses) in the high calcium and atropine without and with dynorphin. C, changes in the relative amplitude of the i.p.s evoked in the high calcium, atropine and dynorphin.
to remove the cholinergic modulation on the NANC inhibitory system in the duodenal preparation. Therefore, these results suggest that dynorphin has an excitatory action on the NANC inhibitory system.

On the opiate receptors for dynorphin, Goldstein et al. (1979, 1981) observed the existence of highly specific dynorphin receptors in the guinea-pig myenteric plexus-longitudinal preparations and Wüster et al. (1981) proposed that dynorphin interacts with κ-opiate receptors. The interaction between dynorphin and κ-opiate receptors was suggested by many investigators (Huidobro-Toro et al., 1981; Oka et al., 1982; Yoshimura et al., 1982).

The release of the NANC inhibitory neurotransmitter was dependent on the external calcium concentrations (Holman and Weinrich, 1975; Ohkawa, 1984). Dynorphin increased the amplitude of the i.p.s evoked in the high calcium solution including atropine but reduced it in the low calcium solution including atropine. These results suggest that the mechanism for increasing the amplitude of the i.p.s by dynorphin and atropine differs from that by the high calcium solution and that the release of the NANC inhibitory transmitter is mainly dependent on the external calcium concentration, and dynorphin may play a subtle role in the release of transmitter.

Dynorphin increased the frequency of the spontaneous action potentials without changes in the resting membrane potential level of the longitudinal smooth muscle. Furthermore, as the excitatory action of dynorphin was not blocked by atropine, the excitatory action of dynorphin may be a direct action on the longitudinal smooth muscle and non-muscarinic excitatory.

Wüster et al. (1980) found opiate receptors with high selectively for dynorphin in the mouse vas deferens and the rabbit ileum. Although it is accepted that the mechanical activity of duodenum is increased by dynorphin, Huidobro-Toro and Yoshimura (1983) have reported that dynorphin caused a small reduction in the spontaneous pendular movements of the rabbit ileum.

This raises the question of why dynorphin decreased the amplitude of the i.p.s elicited in the absence of atropine. The details of this mechanism are not clear. Some possibilities of this mechanism are considered; an interaction between activation of opiate receptors and cholinergic inhibition, an excitation of cholinergic neurons by dynorphin, and two antagonistic opiate receptors in the NANC inhibitory nerves. Further study on these possibilities is being carried out in our laboratory.

REFERENCES


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