Effects of Enkephalin and Endorphin on the Inhibitory Junction Potentials in the Duodenal Smooth Muscle Cells of the Guinea-pig

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Abstract Inhibitory junction potentials (i.j.p.s) evoked by field stimulation were recorded from the smooth muscle cells of the guinea-pig duodenum intracellularly. The membrane potential was $-54.3\,\text{mV}$. The parameters of the i.j.p. were as follows: latency, $71\,\text{msec}$; time to peak, $146\,\text{msec}$; amplitude, $15.5\,\text{mV}$; rate of hyperpolarization, $107\,\text{mV/sec}$; and half decay time of the i.j.p., $193\,\text{msec}$. Met-enkephalin ($10^{-7}-10^{-6}\,\text{M}$) had no effect on the membrane potential and the i.j.p. The membrane potential was decreased by $\beta$-endorphin ($1.7\times10^{-7}-6.8\times10^{-7}\,\text{M}$). Increase in the latency and the time to peak and decrease in the amplitude and the rate of hyperpolarization of the i.j.p. were observed for $\beta$-endorphin. "Spontaneous" excitatory junction potentials (e.j.p.s) were generated by $\beta$-endorphin. Naloxone ($3.1\times10^{-6}-3.1\times10^{-4}\,\text{M}$) hyperpolarized the membrane of the muscle cells. At high concentrations of naloxone ($3.1\times10^{-4}$ and $3.1\times10^{-3}\,\text{M}$), inhibition of the i.j.p. was observed. Levallophan ($2.3\times10^{-4}\,\text{M}$) prolonged the latency and the time to peak and reduced the amplitude of the i.j.p. The membrane potential was slightly decreased by levallophan. "Spontaneous" e.j.p.s were generated by levallophan in a certain population of the cells. It is concluded that Met-enkephalin does not contribute to the non-adrenergic inhibitory transmission and that $\beta$-endorphin acts as a modulator in the control mechanism of the intestinal motility. The effects of naloxone and levallophan on the i.j.p. are discussed.

Key Words: non-adrenergic inhibitory transmission, inhibitory junction potential, opioids, duodenal smooth muscle.

It has been established that intestinal motility is controlled by non-adrenergic inhibitory innervation (BURNSTOCK, 1981). When a brief pulse was given, an inhibitory junction potential was evoked in the smooth muscle cell of the intestinal tract (BURNSTOCK et al., 1966; FURNESS, 1969; TOMITA, 1972; BYWATER et al., 1983).
Successive inhibitory junction potentials evoked by repeated stimulation inhibited the generation of spontaneous action potentials (Bennett et al., 1966; Bauer and Kuriyama, 1982; Ohkawa, 1983). Recently, various neuropeptides have been demonstrated to exist in the intestinal tract and the myenteric neurons (Said and Mutt, 1970; Elde et al., 1976; Larsson et al., 1976; Larsson, 1977; Jessen et al., 1980), and the release of an opiate-like material on electrical stimulation has been reported (Waterfield and Kosterlitz, 1975; Schulz et al., 1977; Puig et al., 1977). On the other hand, the effects of some neuropeptides on the neural activity of the myenteric plexus have been reported (North and Williams, 1976; North et al., 1979; Williams and North, 1979a, b).

The possibility that these neuropeptides are neurotransmitters or neuromodulators is raised (Bryant et al., 1976; Polak et al., 1978; Furness and Costa, 1980). However, it is unlikely that vasoactive intestinal peptide is the non-adrenergic inhibitory transmitter in the guinea-pig intestine (Johnes, 1978; MacKenzie and Burnstock, 1980).

The present experiments, using intracellular techniques to record from the smooth muscle cells of the guinea-pig duodenum, have been carried out in order to investigate whether opiate substances relate to non-adrenergic inhibitory transmission.

**MATERIALS AND METHODS**

Guinea-pigs of either sex weighing 300–400 g were stunned, bled and a short segment (~3 cm from the pylorus of stomach) of duodenum removed. The duodenum was opened along the mesenteric border and a full-thickness strip (4 mm x 2 mm) cut parallel to the longitudinal axis. The strips were mounted serosal surface uppermost in an organ bath with approximately equal lengths of tissue being placed between the stimulating electrodes. Inhibitory junction potentials were recorded in response to rectangular pulses (0.3 msec duration at constant strength) with intracellular micro-electrodes placed less than 1 mm from one of the stimulating electrodes. The electrode used for stimulating the preparation consisted of a pair of silver-silver chloride wires 3 mm apart. Micro-electrodes were inserted into the preparation from the serosal side.

The modified Krebs solution contained (mM): NaCl, 122; KCl, 4.9; NaHCO₃, 15.5; KH₂PO₄, 1.2; CaCl₂, 2.5; MgCl₂, 1.2; and glucose, 11.5. The following drugs were used; β-endorphin (Peptide Institute, Protein Res. Foundation), Met-enkephalin (Peptide Institute, Protein Res. Foundation), Levallophan tartrate (Lorfan, Takeda), and Naloxone (Endo Lab., Inc.). These drugs were generally perfused for 30 min. Values of the measured parameters of muscle membrane and inhibitory junction potential were expressed as the mean±S.E. (n=number of
penetrations of the micro-electrode or number of observed i.j.p.s and e.j.p.s). Statistical significance was assessed using Student’s t-test.

RESULTS

General features of the inhibitory junction potential

The membrane potential of the duodenal smooth muscle cells measured in normal solution was $-54.3 \pm 0.5$ mV (131 cells in 18 preparations). A single stimulus evoked an inhibitory junction potential (i.j.p.). The parameters of the i.j.p. were as follows: the latency was $71.0 \pm 0.9$ msec ($n=152, 43$ cells in 14 preparations) and the time to peak of the i.j.p. was $146.2 \pm 1.2$ msec ($n=152$). The amplitude was $15.5 \pm 0.4$ mV ($n=152$) and the maximum amplitude, 27 mV. The rate of hyperpolarization of the i.j.p. was $107.2 \pm 2.7$ mV/sec ($n=152$). The half decay time was $192.6 \pm 2.5$ msec ($n=152$). Figure 1 shows examples of the evoked i.j.p.s in normal solution.

The parameters of the i.j.p. obtained from one impaled cell had wide variation even under the same conditions of stimulation. The reason for this observation lies in the distribution of the nerve terminals and the histological relationship between the nerve terminals and the duodenal smooth muscle cells. In preliminary experiments, it was found that the parameters of the i.j.p. obtained in the presence of atropine ($2.9 \times 10^{-6}$ M) were not significantly different from those in normal solution. Therefore, the present experiments were carried out in the absence of atropine.

The existence of a relationship between the membrane potential of a cell and the maximum amplitude of the i.j.p. evoked by a single stimulus in the same cell indicated the maximum amplitude of the i.j.p. was decreased with increasing cell membrane potential. The maximum membrane potential level of the i.j.p. exceeded $-80$ mV.

In the 224 cells in normal and test solutions, the i.j.p.s did not occur spontaneously. In 78 cells of the 79 cells observed in normal solution, the excitatory junction potential (e.j.p.) evoked by electrical stimulation was not observed even in the absence of atropine. In one cell (1 cell/79 cells; 1.3%), the “spontaneous” e.j.p.s occurred (Fig. 1B). The amplitude of the e.j.p.s ranged from 2.5 to 4.4 mV and the time to peak of the e.j.p. was about 64 msec. The total duration of the e.j.p. was about 280 msec. The frequency of the “spontaneous” e.j.p. was about 0.9/sec, this activity not continuing for a long period. When a single stimulus was applied to the cell in which the “spontaneous” e.j.p. occurred, an i.j.p. with an amplitude of 19–20 mV was evoked but an evoked e.j.p. was not seen.

Figure 1C shows the i.j.p.s evoked by repeated stimulation at 1 Hz. The successive i.j.p.s evoked at 1 Hz were continued for a long period without reducing the amplitude. When a high frequency of stimulation (12 Hz) was applied, the
hyperpolarization due to successive i.j.p.s reached a maximum level within 3–4 pulses. The magnitude of the hyperpolarization then gradually decreased toward the resting membrane potential level during the stimulation (Fig. 3B and Fig. 5B). The post-stimulus depolarization (p.s.d.) due to an i.j.p. was usually observed, but not always, even under the same conditions of stimulation and even in the same cell. In some cells, action potentials were generated on the p.s.d. due to an i.j.p. Potentiation of the p.s.d. due to successive i.j.p.s was observed.

**Effects of enkephalin on the i.j.p.**

The membrane potentials were $-53.6 \pm 1.4$ mV (26 cells in 4 preparations) in normal solution and $-54.8 \pm 0.9$ mV (21 cells in 2 preparations) at $10^{-7}$ M enkephalin; $-58.0 \pm 0.9$ mV (23 cells in 2 preparations) in normal solution and $-58.9 \pm 1.2$ mV (23 cells in 2 preparations) at $5 \times 10^{-7}$ M enkephalin, and $-54.3 \pm 0.9$ mV (24 cells in 2 preparations) in normal solution and $-54.7 \pm 1.0$ mV (27 cells in 2 preparations) at $10^{-6}$ M enkephalin. There were no significant differences between the mean values in control and enkephalin (Fig. 2A).

The parameters for the control i.j.p.s and the i.j.p.s obtained with enkephalin ($10^{-7}$–$10^{-6}$ M) are summarized in Fig. 2 (B–F). At the concentration of $10^{-6}$ M, the time to peak and the half decay time of the i.j.p. were shortened while there were no significant differences in the latency and the amplitude of the i.j.p. It seems that enkephalin has no effect on the i.j.p.

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Fig. 2. Effects of enkephalin on the membrane potential and the parameters of the i.j.p. in the duodenal smooth muscle. A, membrane potentials in control and enkephalin ($10^{-7}$–$10^{-8}$ M). The observed number is indicated in the text. B–F, changes in the parameters of the i.j.p. in enkephalin. $n$ indicates the number of the observed i.j.p.s obtained from 8–15 cells in 2–4 preparations.

in enkephalin are shown in Fig. 3A.

The maximum amplitude of successive i.j.p.s in response to repeated stimulation (12 Hz) was not affected by enkephalin ($10^{-7}$–$10^{-8}$ M). The mean amplitudes were $14.3\pm1.5$ mV ($n=6$) in normal solution and $16.9\pm1.2$ mV ($n=3$) in $5\times10^{-7}$ M enkephalin, and $19.2\pm1.5$ mV ($n=5$) in normal solution and $19.6\pm1.4$ mV ($n=8$) in $10^{-6}$ M enkephalin. Figure 3B shows the successive i.j.p.s in enkephalin.

Effects of $\beta$-endorphin on the i.j.p.

The membrane potentials were $-60.3\pm0.6$ mV (27 cells in 4 preparations) in normal solution and $-53.7\pm0.2$ mV (26 cells in 2 preparations, $p<0.001$), $-50.8\pm1.4$ mV (26 cells in 2 preparations, $p<0.001$), and $-50.4\pm1.2$ mV (29 cells in 2 preparations, $p<0.001$) at $1.7\times10^{-8}$, $1.7\times10^{-7}$, and $6.8\times10^{-7}$ M, $\beta$-endorphin, respectively. Thus, the membrane potential was decreased by $\beta$-endorphin and the magnitude of the depolarization depended on the concentration of $\beta$-endorphin (Fig. 4A).
Fig. 3. Effects of enkephalin on the i.j.p.s in the duodenal smooth muscle. A (1-3), examples of the i.j.p.s in normal solution and enkephalin ($10^{-7}$–$10^{-6}$ M); B (1-2), successive i.j.p.s evoked by repeated stimulation (12 Hz) in normal solution and enkephalin ($10^{-7}$–$10^{-6}$ M).

Fig. 4. Effects of β-endorphin on the membrane potential and the parameters of the i.j.p. in duodenal smooth muscle. A, membrane potentials in normal solution and β-endorphin. The observed number is indicated in the text. B-F, changes in the parameters of the i.j.p. evoked in β-endorphin. n indicates the number of the observed i.j.p.s obtained from 11-18 cells in 2-4 preparations.
The parameters of the i.j.p.s obtained with $\beta$-endorphin are summarized in Fig. 4B-F. Considerable changes in the parameters were observed at the concentrations of $1.7 \times 10^{-7}$ and $6.8 \times 10^{-7}$ M. The latency and the time to peak of the i.j.p. were prolonged significantly ($p<0.001$). The amplitude of the i.j.p. decreased ($p<0.001$) and the decrease in the amplitude depended on the concentration of $\beta$-endorphin. The mean values of the amplitude were $14.1\pm0.6$ mV ($n=33$, 18 cells in 4 preparations) in normal solution and $13.0\pm0.4$ mV ($n=35$, 18 cells in 2 preparations, no significance) at $1.7 \times 10^{-8}$ M $\beta$-endorphin, $10.9\pm0.6$ mV ($n=23$, 17 cells in 2 preparations, $p<0.001$) at $1.7 \times 10^{-7}$ M $\beta$-endorphin, and $9.4\pm0.6$ mV ($n=32$, 11 cells in 2 preparations, $p<0.001$) at $6.8 \times 10^{-7}$ M $\beta$-endorphin. The rate of hyperpolarization was strongly reduced by $1.7 \times 10^{-7}$ and $6.8 \times 10^{-7}$ M $\beta$-endorphin ($p<0.001$). Decrease in the half decay time of the i.j.p. was also observed at $6.8 \times 10^{-7}$ M $\beta$-endorphin ($p<0.001$). Figure 5A shows examples of the i.j.p.s caused by single stimuli in $\beta$-endorphin.

The maximum amplitude of the hyperpolarization caused by the repetitive stimulation (4 and 12 Hz) was decreased with $\beta$-endorphin ($1.7 \times 10^{-7}$ and $6.8 \times 10^{-7}$ M). At 12 Hz stimulation, the mean amplitudes of the maximum hyperpolarization were $18.2\pm1.3$ mV (5 cells in 2 preparations) in normal solution, $13.5\pm0.3$ mV (4 cells in 2 preparations), $12.3\pm1.3$ mV (3 cells in 2 preparations), and $14.6\pm0.9$ mV (9 cells in 2 preparations) at $1.7 \times 10^{-8}$, $1.7 \times 10^{-7}$, and $6.8 \times 10^{-7}$ M $\beta$-endorphin, respectively. The successive i.j.p.s in $\beta$-endorphin are shown in Fig. 5B.
In β-endorphin (1.7 x 10^-7 and 6.8 x 10^-7 M), 5 cells in 26 cells observed "spontaneous" e.j.p.s but the activity continued for a short period, especially at the initial stage of the perfusion of β-endorphin (Fig. 5C). The amplitudes of e.j.p.s were 3.3±0.3 mV (n=8) at 1.7 x 10^-7 M and 4.7±0.2 mV (n=4) at 6.8 x 10^-7 M β-endorphin. The amplitude of the e.j.p.s was similar to that observed in normal solution. However, in the cells used here, evoked e.j.p.s were not observed. Figure 5C-2 shows the evoked i.j.p. and the "spontaneous" e.j.p. in the same cell. The percentage (5 cells/26 cells; 19%) of the "spontaneous" e.j.p.-producing cells was higher than that in normal solution. In these cells, abortive action potentials were observed at the initial stage of the perfusion of β-endorphin.
Effects of naloxone on the i.j.p.

The mean values of the membrane potentials were \(-52.6 \pm 1.0\) mV (35 cells in 4 preparations) in normal solution, \(-55.9 \pm 1.0\) mV (13 cells in 2 preparations) at \(3.1 \times 10^{-8}\) M naloxone, \(-57.6 \pm 1.2\) mV (13 cells in 2 preparations, \(p<0.001\)) at \(3.1 \times 10^{-6}\) M naloxone, and \(-59.6 \pm 1.0\) mV (24 cells in 2 preparations, \(p<0.001\)) at \(3.1 \times 10^{-4}\) M naloxone. Thus, naloxone (\(3.1 \times 10^{-8}-3.1 \times 10^{-4}\) M) increased the membrane potential (Fig. 6B). At the concentration of \(3.1 \times 10^{-3}\) M naloxone, the membrane potential was \(-52.5 \pm 0.6\) mV (8 cells in 2 preparations).

Figure 6A shows examples of the i.j.p.s evoked by single stimuli in naloxone (\(3.1 \times 10^{-8}-3.1 \times 10^{-3}\) M). Significant effects of naloxone on the i.j.p. were observed at the concentrations of \(3.1 \times 10^{-4}-3.1 \times 10^{-3}\) M. The parameters of the i.j.p. evoked in naloxone are summarized in Fig. 6B. The latency and the time to peak of the i.j.p. were prolonged \((p<0.01\) and \(p<0.001\)). The rate of hyperpolarization of the i.j.p. was strongly reduced \((p<0.001)\). A decrease in the
amplitude of the i.j.p. was observed \((p<0.001)\).

**Effects of levallorphan on the i.j.p.**

Levallorphan \((2.3 \times 10^{-5} - 2.3 \times 10^{-4} \text{ M})\) slightly decreased the membrane potential; \(-53.7 \pm 0.8 \text{ mV}\) (40 cells in 4 preparations) in normal solution, \(-50.3 \pm 1.3 \text{ mV}\) (27 cells in 2 preparations) at \(2.3 \times 10^{-5} \text{ M}\), and \(-47.5 \pm 1.6 \text{ mV}\) (20 cells in 2 preparations \(P<0.005\)) at \(2.3 \times 10^{-4} \text{ M}\) (Fig. 7).

Examples of the i.j.p.s evoked by single stimuli in levallorphan \((2.3 \times 10^{-5} \text{ and } 2.3 \times 10^{-4} \text{ M})\) are shown in Fig. 8A. Changes in the parameters of the i.j.p. with levallorphan \((2.3 \times 10^{-4} \text{ M})\) are shown in Fig. 7. The latency and the time to peak of the i.j.p. were prolonged significantly \((p<0.001)\). The amplitude and the rate of hyperpolarization of the i.j.p. were decreased \((p<0.001)\). The mean amplitudes of the maximum hyperpolarization due to repeated stimulation (12 Hz) were \(18.9 \pm 2.3 \text{ mV}\) (4 cells in 2 preparations) in normal solution and \(13.6 \pm 2.9 \text{ mV}\) (5 cells in 2 preparations) at \(2.3 \times 10^{-4} \text{ M}\) levallorphan (Fig. 8B).

“Spontaneous” e.j.p.s were generated in levallorphan \((2.3 \times 10^{-5} \text{ and } 2.3 \times 10^{-4} \text{ M}, \text{ Fig. 9A})\). At the concentration of \(2.3 \times 10^{-4} \text{ M}\), “spontaneous” e.j.p.s were generated in 6 cells (26\%) in 23 cells observed. This percentage was higher than that in normal solution. The amplitude of the “spontaneous” e.j.p.s was
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10.2±0.4 mV (n=14) and was larger than that (up to 4.4 mV) in normal solution. Total duration of the e.j.p. ranged from 160 to 280 msec.

When a single pulse was given in 2.3×10^{-4} M levallorphan, most cells generated an i.j.p. only. However, an e.j.p. followed by an i.j.p. was evoked in 4 cells (Fig. 9B). In these cells, the interval between the stimulation and the peak of the i.j.p. was slightly prolonged. The intervals between the stimulus and the peak of the i.j.p. were 212.3±2.6 msec (7 cells, n=40) in normal solution, 276.4±6.5 msec (7 cells, n=22) without the e.j.p. in levallorphan, and 315.4±4.8 msec (4 cells, n=17) with the initial e.j.p. in levallorphan. When the e.j.p. was evoked, an action potential was sometimes triggered on the e.j.p. (Fig. 9B).

DISCUSSION

In the colon, it has been reported that enkephalin has a strong inhibitory effect on the amplitude of the i.j.p., and it has been suggested that opiates, enkephalin and morphine might act either on preganglionic nerve endings or on non-adrenergic, non-cholinergic cell bodies (BLANQUET et al., 1982). However, in the present experiments, enkephalin had no significant effect on the membrane po-
tential and the evoked i.j.p. in duodenal smooth muscles. "Spontaneous" e.j.p.s did not appear in enkephalin but did appear in endorphin. The results suggest that enkephalin does not contribute to non-adrenergic inhibitory neurotransmission.

In the guinea-pig ileum, it was found that enkephalin inhibited the activity of the myenteric neurons and decreased the output of acetylcholine (NORTH and WILLIAMS, 1976; WATERFIELD et al., 1977; NORTH et al., 1979; WILLIAMS and NORTH, 1979a). These effects of enkephalin on the myenteric neurons seem not to be related to the non-adrenergic inhibitory transmission, because of the lack of effect of enkephalin on the evoked i.j.p.s. A correlation between the electrical activity of the myenteric neurons and the i.j.p. in the intestinal wall is unknown.

The membrane of the duodenal smooth muscle was depolarized and the evoked i.j.p.s were suppressed by β-endorphin while "spontaneous" e.j.p.s were generated. The prolongation in the latency and the time to peak and the decrease in the amplitude of the evoked i.j.p. were significant.

Nevertheless an increase in the amplitude of the evoked i.j.p. due to the depolarization by β-endorphin is expected; the amplitude of the i.j.p. decreased at high concentrations of β-endorphin. A simple explanation is that the changes in the i.j.p. are caused by a decrease in the amount of the non-adrenergic inhibitory substance released. In the ileal smooth muscle, the i.j.p. amplitude decreased markedly on reducing the external calcium ion concentration (HOLMAN and WEINRICH, 1975). Therefore, it is speculated that β-endorphin inhibits the utilization of Ca^{2+} for releasing the chemical transmitter. On the generation of the "spontaneous" e.j.p. in β-endorphin, it is considered that the excitatory neurons are activated directly or that inputs to the excitatory neurons from other elements in the duodenal wall are increased by β-endorphin.

There is a possibility that β-endorphin is not a substance concerned with non-adrenergic inhibitory transmission but acts as a modulator on the control mechanism of intestinal motility.

The effects of naloxone and levallorphan on the evoked i.j.p. in duodenal smooth muscle were investigated since these agents have an antagonistic effect on actions of morphine and opioid peptides. These agents suppressed the amplitude and the rate of hyperpolarization of the i.j.p. and prolonged the latency and the time to peak of the i.j.p.

ITO and TAJIMA (1980) have reported that morphine does not abolish the i.j.p. in guinea-pig ileum. However, there remains the possibility that morphine-like substances contribute to the non-adrenergic inhibitory pathway. The inhibitory effect of naloxone on the i.j.p. seems not to be related to myenteric neuron activity because naloxone has no effect to this activity (NORTH and WILLIAMS, 1976). Direct inhibitory actions of naloxone and levallorphan on the non-adrenergic inhibitory nerves cannot be excluded.

A small depolarization in the duodenal smooth muscle caused by levallorphan
was observed and “spontaneous” e.j.p.s were generated by levallorphan. The excitability in the excitatory neurons seems to be affected by levallorphan because the evoked e.j.p. which did not normally occur in normal solution was frequently observed in levallorphan.

Further experiments will be required to clarify what substances contribute to non-adrenergic inhibitory neurotransmission and how the i.j.p. and e.j.p. relate to the activity of myenteric neurons in the intestinal tract.

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Japanese Journal of Physiology