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EFFECT OF OPIOID PEPTIDES ON CIRCULAR MUSCLE OF CANINE DUODENUM

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SUMMARY

1. The effects of opioid peptides on inhibitory transmission in the circular muscle layer of canine duodenum were investigated *in vitro* using simultaneous mechanical and intracellular electrical recording techniques.

2. Exogenously added [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin (1–13) decreased the amplitude of non-adrenergic, non-cholinergic inhibitory junction potentials (IJPs) evoked by transmural nerve stimulation.

3. A selective δ-receptor agonist, DPDPE ([D-Pen^2, D-Pen^5]enkephalin), and a selective μ-receptor agonist, PL017 (Try-Pro-NMePhe-D-Pro-NH_2), decreased the amplitude of IJPs whereas a selective κ-receptor agonist, U-50,488H ([trans-3,4-dichloro-*N*-methyl-*N*-(2-91-pyrolidinyl)-cyclohexyl]-benzeneacetamide methanesulphonate), in large doses (1 μM) produced only a small reduction.

4. A selective δ-receptor antagonist, ICI-174,864, blocked the effect of DPDPE but not that of PL017 suggesting the presence of distinct δ- and μ-opioid receptors on inhibitory motor nerves.

5. Exogenously added dynorphin (1–13) decreased the amplitude of IJPs. δ-Opioid receptors appeared to be involved because ICI-174,864, a selective δ-antagonist, blocked the inhibitory effect of exogenously added dynorphin (1–13).

6. The inhibitory effect of the opioid peptides was still observed in preparations of circular muscle devoid of myenteric and submucosal plexuses, indicating that the site of action was on inhibitory motor nerve fibres located within the circular muscle layer and not on neuronal cell bodies in the enteric plexuses.

7. It was concluded that in the canine small intestine, opioid peptides could modulate release of inhibitory transmitter(s) at or near nerve terminals of inhibitory motor nerves innervating circular muscle cells.

INTRODUCTION

The endogenous opioid peptides [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin are present in ganglion cells of the myenteric and submucosal ganglia and in axon varicosities in both muscle layers of the small and large intestine of several vertebrates including man and dog (Polak, Sullivan, Bloom & Facer, 1977; Alumets, Håkanson, Sundler & Chang, 1978; Larsson, Childers & Snyder, 1979; Schultzberg,

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Hökfelt, Nilsson, Terenius, Rehfeld, Brown, Elde, Goldstein & Said, 1980; Daniel, Costa, Furness & Keast, 1985; Spampinato, Ferri, Candeletti, Romualdi, Civicchini, Soimero, Labo & Ferri, 1988). The opioid peptide-containing nerves present in the circular muscle layer originate from cell bodies in the myenteric plexus because they are still present following extrinsic denervation but disappear from the circular muscle layer following surgical removal of the overlying longitudinal muscle layer and myenteric plexus (Furness, Costa & Miller, 1983; Costa, Furness & Cuello, 1985).

The results from a number of studies suggest that [Met^5]enkephalin, [Leu^5]-enkephalin and dynorphin function as neurotransmitters in the gut. Thus, [Met^5]enkephalin inhibits electrically evoked release of prelabelled pools of acetylcholine (Vizi, Ono, Adam-Vizi, Duncalf & Foldes, 1984). The enkephalins also act postsynaptically to hyperpolarize the soma membrane of enteric neurone (North, Katayama & Williams, 1979). During peristaltic activity in the guinea-pig small intestine and rat large intestine, there is a decrease in the release of endogenous opioid peptides during the inhibitory phase of the peristaltic reflex and an increase during the excitatory phase (Clark & Smith, 1981; Donnerer, Bartho, Holzer & Lembeck, 1984; Grider & Makhlouf, 1987*a*).

Recent biochemical studies of the distribution of μ-, δ- and κ-receptor types show that μ- and δ-receptors are located on axonal varicosities present in the circular muscle layer of the canine intestine (Allescher, Ahmad, Kostka, Kwan & Daniel, 1989) suggesting opioid control of motility also takes place near or at nerve terminals innervating the muscularis externa. The purpose of this study was to determine the functional significance of this arrangement of opioid receptors. Parts of this work have been previously communicated as abstracts (Bauer & Szurszewski, 1988, 1989).

METHODS

Adult mongrel dogs (17–40 kg) were anaesthetized with an intravenous injection of sodium pentobarbitone (30 mg/kg). The abdomen of each animal was opened by a mid-line incision and the duodenum removed and immersed in a preoxygenated Krebs solution. Animals were killed by an overdose of sodium pentobarbitone administered intravenously following removal of a number of smooth muscle-containing organs for studies by other investigators. After cutting along the mesenteric border of the duodenum, a section of proximal duodenum (5 cm in length) beginning 10 cm distal to the gastroduodenal junction was removed and placed in a dissecting dish. The mucosa was removed and strips of full thickness muscularis externa (1 × 8 mm) were prepared by cutting parallel to the circular muscle layer. Each muscle strip was placed in a recording chamber with the cross-sectional face upward. One end (1 × 3 mm) was pinned down to record intracellular electrical activity and the other end attached to an isometric force transducer to record mechanical activity. Cross-sectional pinning exposed the entire thickness of the circular muscle layer and allowed for consistent impalement of cells within 0·5 mm of the myenteric plexus (Bauer, Reed & Sanders, 1985). In some experiments, strips of circular muscle (1 × 8 mm) from the middle third of the circular muscle layer were prepared by removing and discarding the longitudinal muscle layer, myenteric plexus and approximately one-third of the attached circular muscle and the submucosa, submucosal plexus and approximately one-third of the attached circular muscle. The remaining circular muscle was also pinned in cross-section with one end pinned down for intracellular recording and the other end for recording isometric tension. Recordings of intracellular electrical activity were obtained from muscle cells located approximately halfway through the thickness of this isolated circular muscle preparation.

Following equilibration and determination of L_0 (length at which contraction amplitude was a maximum) as described previously (Hara & Szurszewski, 1986), smooth muscle cells were impaled with glass microelectrodes (20–50 MΩ) filled with 3 M-KCl. Recordings were accepted when a sharp

change in voltage of greater than −60 mV was observed. Stable impalements were maintained for up to 5 h. Intracellularly recorded potentials were amplified (WPI model 701) and displayed on an oscilloscope (Tektronix 5113). Electrical signals and mechanical activity were recorded on an FM tape-recorder (Hewlett-Packard 3964A) for off-line analysis. Tetrodotoxin-sensitive responses were evoked by transmural stimulation using two platinum wires placed parallel to the long axis of the preparation. Data were compiled as means ± standard error of the mean (S.E.M.) and were analysed for statistical significance using an unpaired *t* test.

The modified Krebs solution used in this study contained (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·5; Mg^{2+}, 1·2; Cl^-, 134; HCO_3^-, 15·5; $H_2PO_4^-$, 1·2; and glucose, 11·5, and was gassed with 97% O_2–3% CO_2 to maintain a pH of 7·4. The temperature of the recording chamber was maintained at 38 ± 0·5 °C.

Drugs used were atropine sulphate, cyclic [D-Pen², D-Pen⁵]enkephalin (DPDPE), dynorphin (1–13), [Leu⁵]enkephalin, [Met⁵]enkephalin, tetrodotoxin (all obtained from Sigma); Try-Pro-NMePhe-D-Pro-NH_2 (PL017) (Peninsula); [trans-3,4-dichloro-*N*-methyl-*N*-(2-91-pyrolidinyl)-cyclohexyl]-benzeneacetamide methanesulphonate (U-50,488H; Upjohn); ICI-174,864 (Cambridge). Concentrations of the substances given in the Results are the final concentration perfusing the chamber.

RESULTS

General observations

The mean maximum resting membrane potential recorded between spontaneous electrical slow wave activity in circular smooth muscle cells was −69 ± 1·8 mV ($n = 18$). Spontaneously slow waves occurred at a frequency of 12·8 ± 1·3 cycles min^{-1} ($n = 18$). Their mean amplitude and duration were 12 ± 1·3 mV ($n = 8$) and 789 ± 55 ms ($n = 12$), respectively. A monophasic contraction was associated with each slow wave.

Exogenously added [Met⁵]enkephalin, dynorphin (1–13) and the selective μ-opioid agonist, PL017 (each tested five times in five different preparations at a concentration of 1 μM), had no detectable effect on tone, amplitude or frequency of monophasic contractions and had no effect on intracellularly recorded electrical activity including resting membrane potential and slow wave amplitude and frequency.

In preparations consisting of the middle third of the circular muscle layer, the resting membrane potential was −64 ± 2·1 mV ($n = 6$). In all such preparations studied, there were neither spontaneously occurring slow waves nor spontaneous contractions. These findings are consistent with previous observations made in similar preparations of the canine jejunum (Hara, Kubota & Szurszewski, 1986). Exogenously added [Met⁵]enkephalin ($n = 6$) dynorphin (1–13) ($n = 4$) and PL017 ($n = 5$), each at concentrations ranging from 1 to 10 μM, had no detectable effect on either resting membrane potential or muscle tone.

Transmural stimulation with frequencies ranging from 1 to 20 Hz for 1 s was used to evoke tetrodotoxin-sensitive responses in full thickness preparations. Only IJPs were observed. The IJP amplitude was frequency dependent and reached a maximum when frequencies ranging from 15 to 20 Hz were used (cf. Fig. 3). IJPs were unaffected by atropine, phentolamine, propranolol and hexamethonium (each, 10 μM) alone ($n = 3$) or in combination ($n = 3$). Thus, the neurotransmitter that mediated the IJPs was non-adrenergic and non-cholinergic.

Effect of opioids on evoked inhibitory junction potential

[Met⁵]enkephalin

[Met⁵]enkephalin reversibly decreased the amplitude of IJPs (Fig. 1). In the example shown in Fig. 1, the IJP amplitude was 16 mV in normal Krebs solution.

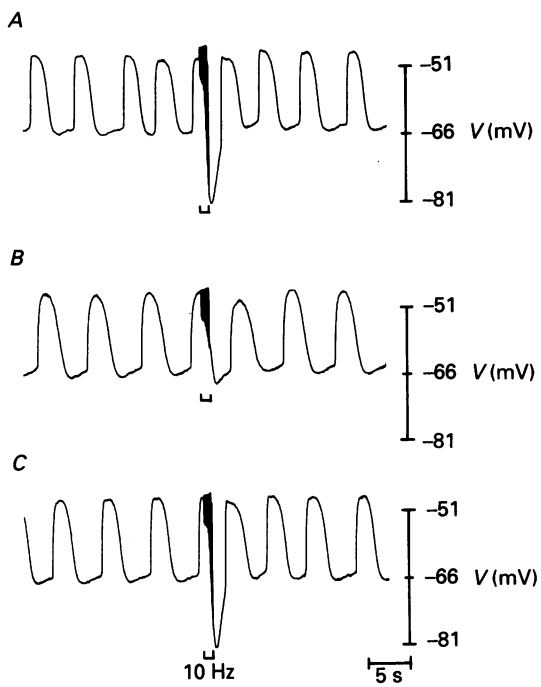

Fig. 1. Effect of [Met⁵]enkephalin on inhibitory junction potentials recorded from circular muscle of canine duodenum. *A*, *B* and *C* show IJPs recorded from the same cell in normal Krebs solution, in the presence of [Met⁵]enkephalin (10 μM) and after a 14 min wash period with Krebs solution, respectively.

Within 1·5 min after adding [Met⁵]enkephalin, the IJP amplitude was 3 mV. Thereafter, there was no further change as long as the agonist was present. Following wash-out of [Met⁵]enkephalin from the tissue, the full amplitude of the IJP was restored. In five other experiments, the mean ($\pm$S.E.M.) IJP amplitude (10 Hz, 1 s) was $17 \pm 2{\cdot}2$ mV in normal Krebs solution and $4 \pm 0{\cdot}6$ mV ($P < 0{\cdot}001$) 3 min after adding [Met⁵]enkephalin (10 μM). The effects of several different concentrations of [Met⁵]enkephalin on IJPs evoked by transmural stimulation at 6 Hz are shown in Fig. 2*A*. Threshold and IC_{50} concentrations were 0·3 nM and 0·13 μM, respectively. In three of the five experiments, complete dose–response curves were obtained while maintaining intracellular recording from the same three cells. In these experiments, IJPs were recorded in normal Krebs solution, in the presence of an opioid peptide until a steady-state effect was achieved and during wash-out until the IJP amplitude returned to its control value ($\sim$ 30 min). Thereafter, the next opioid concentration was tested. In two other experiments, IJPs were recorded from the same two cells in

normal Krebs solution and in solutions containing increasing concentrations of [Met^5]enkephalin without the intervening wash periods. There was no difference in the dose–response relationships obtained by these two approaches, indicating that desensitization did not occur during prolonged exposure required for constructing

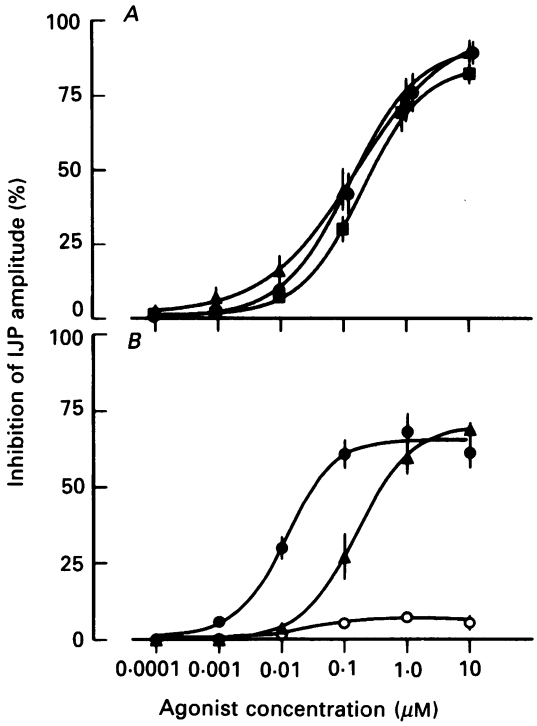

Fig. 2. Dose–response curves for the inhibitory effect of several different opioid receptor agonists on IJP amplitude. In *A*, recordings made from a full thickness preparation. In *B*, recordings made from a preparation devoid of myenteric and submucosal ganglia. Amplitude of IJPs in normal Krebs solution was expressed as 100%. IJPs were evoked by transmural nerve stimulation for a period of 1 s at 6 Hz (0·35 ms pulse duration, 150 V). IJP amplitudes were measured when steady-state effects were observed for each concentration used. Each data point is the mean ± S.E.M. of five (panel *A*) or four (panel *B*) experiments. In *A*: ●, [Met^5]enkephalin; ▲, [Leu^5]enkephalin; ■, dynorphin. In B: ●, DPDPE; ▲, PL017; ○, U-50,488H.

cumulative dose–response curves. The inhibitory effect of [Met^5]enkephalin (1 μM, $n = 4$) was maintained constant even during prolonged periods (> 30 min) of exposure.

The effect of varying the frequency of stimulation on IJP amplitude in normal Krebs solution and in the presence of three different concentrations of [Met^5]-enkephalin is shown in Fig. 3. It can be seen that IJPs evoked at low frequencies of stimulation were more sensitive to the inhibitory effects of all concentrations of [Met^5]enkephalin used than were IJPs evoked at higher frequencies of stimulation. Thus, at 4 Hz, IJP amplitudes were significantly ($P < 0{\cdot}001$) reduced whereas at 20 Hz there was no inhibitory effect of [Met^5]enkephalin (10 μM).

[Leu⁵]enkephalin

As with [Met⁵]enkephalin, [Leu⁵]enkephalin reduced IJP amplitude in a concentration-dependent manner (cf. Fig. 2*A*). Threshold and IC_{50} concentrations were 1 nM and 0·16 μM ($n = 5$), respectively. As with [Met⁵]enkephalin, the

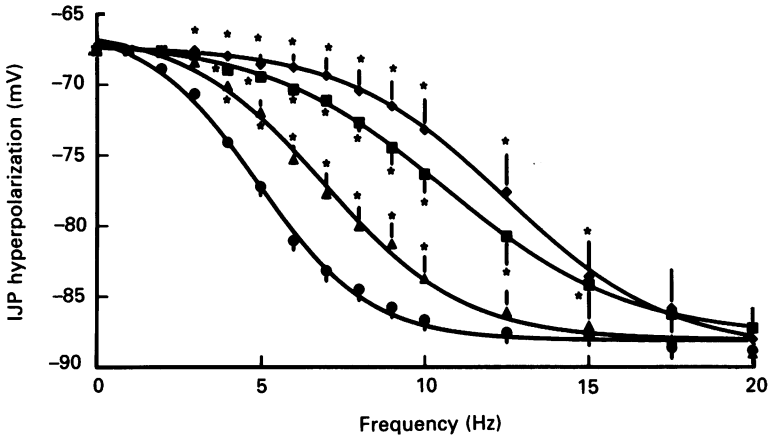

Fig. 3. Effect of three different concentrations of [Met⁵]enkephalin on IJP amplitude evoked by transmural nerve stimulation for 1 s at frequencies from 1 to 20 Hz (0·35 ms pulse duration, 150 V). IJP amplitudes evoked in opioid-containing Krebs solution that were significantly different from those observed in normal Krebs solution (evoked at the same frequency of stimulation) are marked by an asterisk ($P < 0·05$; mean ± S.E.M.). See text for further details. ●, Krebs solution ($n = 18$); ▲, 0·1 μM [Met⁵]enkephalin ($n = 5$); ■, 1·0 μM [Met⁵]enkephalin ($n = 5$); ◆, 10 μM [Met⁵]enkephalin ($n = 5$).

inhibitory effect of [Leu⁵]enkephalin was more marked at low (< 10 Hz) frequencies of stimulation than were IJPs evoked at higher frequencies (> 10 Hz).

Dynorphin (1–13)

Dynorphin-like immunoreactive material is present in nerves in the circular muscle layer as well as in the myenteric plexus of the small intestine (Daniel *et al.* 1985; Spampinato *et al.* 1988). Therefore, the effect of exogenously added dynorphin (1–13) on IJP amplitude was studied. Addition of dynorphin (1–13) to the bathing solution suppressed IJP amplitude (Fig. 2*A*). Threshold and IC_{50} values were 1 nM and 0·2 μM ($n = 5$), respectively. As with the enkephalins, the inhibitory effect of exogenously added dynorphin (1–13) was more marked at low (< 10 Hz) frequencies of stimulation.

Site of action

Opioid peptides inhibit cholinergic transmission in the guinea-pig myenteric and submucosal plexuses by a presynaptic inhibitory mechanism (Egan & North, 1981; Cherubini, Morita & North, 1985; Cherubini & North, 1985; Mihara & North, 1986; North, Williams, Surprenant & Christie, 1987). To determine whether the opioid peptides reduced IJP amplitude in dog duodenum by interfering with cholinergic transmission to inhibitory neurones innervating circular smooth muscle cells, the

effect of [Met^5]enkephalin on IJP amplitude was determined in the absence and presence of nicotinic and muscarinic receptor antagonists. In the absence of both antagonists, [Met^5]enkephalin decreased the amplitude of IJPs from $16 \pm 1{\cdot}8$ to $4 \pm 1{\cdot}5$ mV ($n = 3$). In the presence of both antagonists, [Met^5]enkephalin similarly reduced IJPs from $17 \pm 1{\cdot}1$ mV (mean $\pm$ S.E.M.) to $5 \pm 0{\cdot}9$ mV ($n = 3$). To test the hypothesis that [Met^5]enkephalin may have acted at receptor sites distributed along the inhibitory nerves coursing through the circular muscle layer, the effect of the opioid peptides on IJP amplitude was determined in preparations ($n = 6$) consisting of the middle third of the circular muscle which were devoid of myenteric and submucosal plexuses. Although both plexuses were absent, field stimulation nevertheless evoked tetrodotoxin-sensitive IJPs and [Met^5]enkephalin, [Leu^5]-enkephalin and exogenously added dynorphin (1–13) still reduced IJP amplitude. An example of the inhibitory effect of [Met^5]enkephalin on IJPs in ganglion-free circular muscle is shown in Fig. 4*A*. Similar results were obtained with [Leu^5]enkephalin and exogenously added dynorphin (1–13) (each, $n = 3$). The effect of naloxone (50 μM) is shown in Fig. 4*B*. Although naloxone tested at concentrations ranging from 0·3 to 50 μM had no significant effect on IJP amplitude itself (Fig. 4*B*), it abolished the inhibitory effect of [Met^5]enkephalin (Fig. 4*C*). These data suggested that the enkephalins and exogenously added dynorphin (1–13) inhibited release of an inhibitory neurotransmitter(s) by acting on specific opioid receptors located on projections of inhibitory nerves coursing through the circular muscle layer.

Type of opioid receptors mediating inhibition

Selective, opioid receptor agonists were used to determine which receptor type(s) mediated the presynaptic inhibitory effects described above. DPDPE was used to activate δ-receptors (Mosberg, Hurst, Hruby, Gee, Yamamura, Galligan & Burks, 1983), PL017 to activate μ-receptors (Chang, Wei, Killian & Chang, 1983) and U-50,488H to activate κ-receptors (Vonvoigtlander, Lahti & Ludens, 1983). Preparations of the middle third of the circular muscle layer devoid of the myenteric and submucosal plexuses were used to remove any effect these agonists have on transmission in enteric ganglia. The effect of DPDPE and PL017 on IJP amplitude is shown in Fig. 4*C* and *D*, respectively. Both agonists reduced IJP amplitude. The κ-receptor agonist U-50,488H had no significant effect as shown in Fig. 5*A*. The dose–response relationship for each agonist is shown in Fig. 2*B*. Threshold and IC_{50} concentrations for the δ-agonist DPDPE were 0·3 and 11·5 nM, respectively. Threshold and IC_{50} concentrations for the μ-agonist PL017 were 3 and 158 nM, respectively. DPDPE and PL017 at the IC_{50} concentrations caused a 33 and 34% decrease in IJP amplitude. In contrast to the inhibitory effect of δ- and μ-agonists, high concentrations of the κ-receptor agonist, U-50,488H, decreased IJP amplitude by only $7 \pm 1{\cdot}5\%$ ($n = 5$). The selective opioid agonists used had no effect on resting membrane potential.

To substantiate further the presence of δ- and μ-receptors, ICI-174,864, a selective δ-antagonist (Kosterlitz, 1985), was used to determine if the effect of DPDPE on IJP amplitude could be blocked without interfering with the inhibitory effect of PL017. ICI-174,864 (10 μM) blocked the inhibitory effect of DPDPE ($n = 4$). However, ICI-174,864 had no significant ($P < 0{\cdot}01$) effect on the inhibitory effect of the PL017

($n = 4$). In a series of four experiments, DPDPE (1 μM) decreased IJP amplitude from $15 \pm 2{\cdot}4$ mV in normal Krebs solution to $3 \pm 1{\cdot}7$ mV ($P < 0{\cdot}001$). In the presence of ICI-174,864 (10 μM), however, the inhibitory effect of DPDPE (1 μM) was blocked. The IJP amplitude recorded in ICI-174,864 alone was $15 \pm 2{\cdot}4$ mV and in the

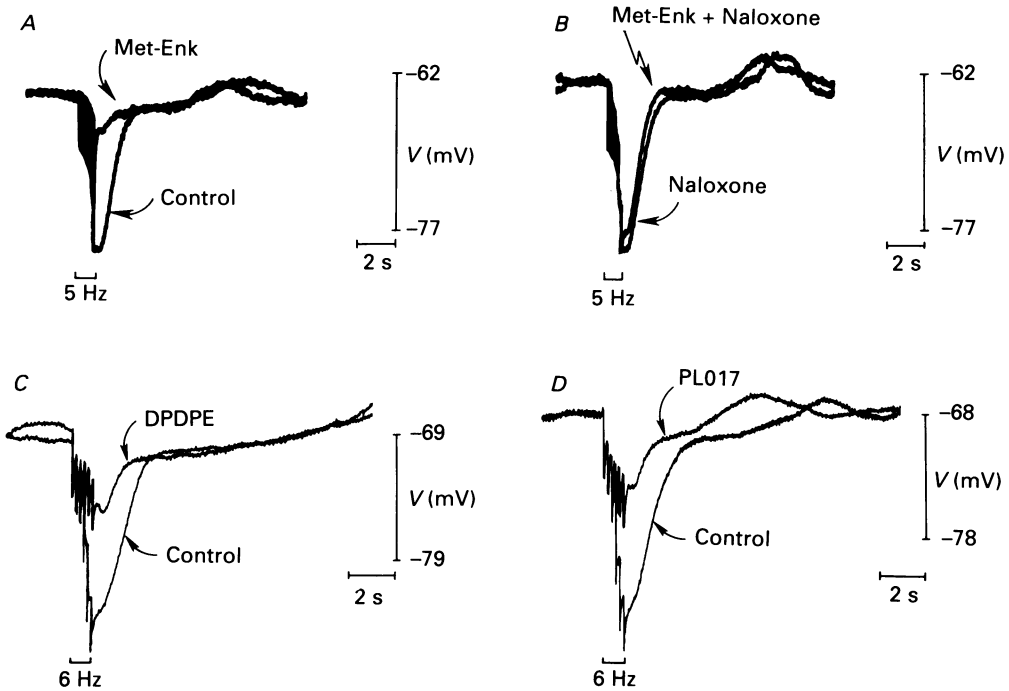

Fig. 4. Upper traces (*A* and *B*) illustrate the effect of [Met^5]enkephalin (Met-Enk) and naloxone on IJPs recorded from the same muscle cell in a preparation that consisted of the middle third of the circular muscle layer devoid of enteric ganglia. *A* shows two superimposed IJPs: one was recorded in normal Krebs solution and the other in the presence of Met-Enk (0·3 μM). *B* shows two superimposed IJPs: one recorded in the presence of naloxone (50 μM) and the other in the presence of Met-Enk (0·3 μM) and naloxone (50 μM). IJPs were evoked by transmural nerve stimulation for a period of 1 s at 5 Hz (0·35 ms pulse duration, 150 V). Lower traces (*C* and *D*) illustrate the effect of selective δ- and μ-opioid receptor agonists on IJP amplitude recorded in a preparation devoid of myenteric and submucosal ganglia. *C* and *D* show two superimposed IJPs: one IJP was recorded in normal Krebs solution (control) and the other either in the presence of DPDPE (1 μM, *C*) or PL017 (1 μM, *D*). IJPs were elicited by transmural nerve stimulation for a period of 1 s at 6 Hz (0·35 ms pulse duration, 150 V).

presence of both the antagonist and DPDPE (1 μM) was $14 \pm 2{\cdot}2$ mV ($P > 0{\cdot}05$). Similar to DPDPE, PL017 (1 μM) decreased the amplitude of the IJP from $15 \pm 1{\cdot}7$ mV in normal Krebs solution to $3 \pm 1{\cdot}7$ mV. However, in the presence of ICI-174,864 the effect of PL017 was not blocked. The IJP amplitude recorded in ICI-174,864 alone was $15 \pm 1{\cdot}8$ mV and in the presence of both the antagonist and PL017 (1 μM) was $2 \pm 1{\cdot}2$ mV ($P < 0{\cdot}001$).

Dynorphin (1–13) is considered to be an endogenous ligand for κ-opioid receptors

(Chavkin, James & Goldstein, 1982; Burks, 1986). As shown in Fig. 5*A*, the specific κ-receptor agonist U-50,488H had no effect on IJP amplitude suggesting that κ-receptors were not present on axons of inhibitory motor nerves. To determine whether exogenously added dynorphin (1–13) acted through either δ- or μ-opioid

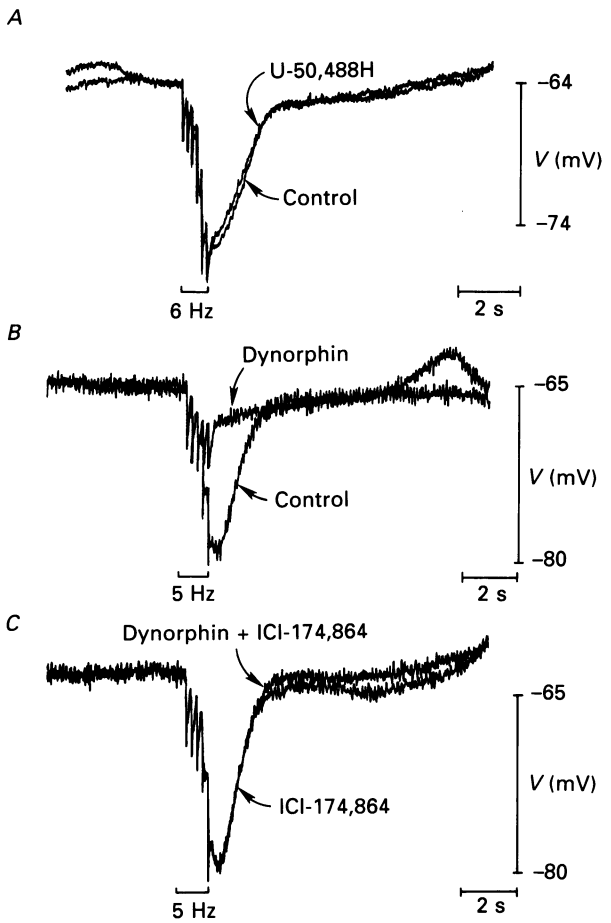

Fig. 5. Effect of U-50,488H (1 μM, *A*), a selective κ-opioid receptor agonist and of exogenously added dynorphin (1–13) (1 μM) before (*B*) and after (*C*) blocking δ-receptors with ICI-174,864. *A* and *B* consist of two superimposed traces: one recorded in normal Krebs solution (control) and the other after adding an agonist. In *C*, one of the two superimposed traces was recorded in the presence of the δ-receptor agonist, ICI-174,864, and the other after adding dynorphin (1–13) to the ICI-174,864-containing solution. These data suggest that κ-receptor mediated effects were absent. IJPs were elicited by transmural nerve stimulation for a period of 1 s at 6 Hz (0·35 ms pulse duration, 150 V). Recordings made from two preparations both devoid of myenteric and submucosal ganglia. Recordings in *A* from one preparation; recordings in *B* and *C* from the same cell of another preparation.

receptors, the effect of adding dynorphin (1–13) was determined before and after blocking δ-receptors with ICI-174,864 (10 μM). It can be seen in Fig. 5*C* that the inhibitory effect of exogenously added dynorphin (1–13) on IJP amplitude was

nearly completely blocked by ICI-174,864. In four other experiments, adding dynorphin (1–13) (1 μM) to the bathing solution decreased IJP amplitude from $14 \pm 0{\cdot}5$ mV (mean $\pm$ S.E.M.) to $3 \pm 0{\cdot}2$ mV (mean $\pm$ S.E.M.; $P < 0{\cdot}001$). When the bathing solution contained ICI-174,864, the mean ($\pm$S.E.M.) IJP amplitude was not significantly changed ($14 \pm 1{\cdot}5$ mV, $P > 0{\cdot}05$). Addition of dynorphin (1–13) in the presence of ICI-174,864 had no significant effect on IJP amplitude ($13 \pm 1{\cdot}6$ mV, mean $\pm$ S.E.M., $P > 0{\cdot}5$). These data suggest that the effect observed after adding dynorphin (1–13) was due to activation of δ-receptors.

To further substantiate that dynorphin (1–13) was acting at δ-receptor sites, the inhibitory effect of dynorphin (1–13) on IJP amplitude was tested in the presence of norbinaltorphimine, a putative selective κ-receptor antagonist (Sander, Portoghese & Gintzler, 1988; Traynor, 1989). In four experiments norbinaltorphimine (0·1–10 μM) caused a concentration dependent decrease in the inhibitory effect of an IC_{50} concentration of dynorphin (1–13). However, increasing concentrations of the antagonist also progressively blocked the inhibitory effects of an IC_{50} concentration of the δ-agonist DPDPE (20 nM) and larger doses of both [Met^5]enkephalin and the μ-receptor agonist PL017 (each 10 μM, $n = 3$ each). In all these experiments, norbinaltorphimine (10 μM) completely blocked the inhibitory effect of all agonists. The pharmacological details of the norbinaltorphimine effect were not studied, but in this tissue norbinaltorphimine indiscriminately blocked opioid receptors at similar concentrations and could not be used as a selective κ-antagonist. Therefore, the antagonist effect of norbinaltorphimine on the inhibitory effect of dynorphin (1–13) cannot be viewed as evidence for an effect of κ-opioid receptors.

DISCUSSION

The present observations show that exogenously added enkephalins and dynorphin (1–13), naturally occurring neuropeptides in enteric nerves, depressed inhibitory neural transmission to circular smooth muscle cells by acting on specific opioid receptors located on axons of inhibitory motor nerves coursing through the circular muscle layer. The site of action of the opioid peptides was on axons and/or presynaptic terminals of inhibitory motor neurones because the opioid peptides still inhibited evoked IJPs in preparations in which the myenteric and submucosal plexuses were removed. No evidence was obtained for the presence of excitatory opioid receptors on circular smooth muscle cells. Although this result is in agreement with those obtained by Allescher *et al.* (1989), it is at variance with those obtained by Bitar & Makhlouf (1985) who obtained evidence for δ-, μ- and κ-receptor types on dissociated smooth muscle cells from guinea-pig and human small intestine. In contrast to this study, Ito & Tajima (1980) reported that morphine, a μ-receptor agonist, caused no suppression of the IJP recorded from circular muscle of guinea-pig ileum. These results could represent a true species difference, but opioids also have been observed to decrease IJP amplitude in human and baboon small intestine (Bauer & Szurszewski, 1989). However, as shown in this study, opioid suppression of the IJP could be missed by using high frequencies of stimulation. In guinea-pig, a single pulse of stimulation causes a maximal membrane hyperpolarization at a strong stimulus intensity (Bywater, Holman & Taylor, 1981). Therefore, the inhibitory

effect of morphine may have been masked by the parameters used by Ito & Tajima (1980) (i.e. either a strong intensity of stimulation or a high frequency of stimulation).

When the results from the present study and previous studies by others are considered together, they indicate that endogenous release of opioid peptides in the canine circular muscle layer would suppress or inhibit release of an inhibitory neurotransmitter thereby increasing contractile activity. Induction of phasic, non-propulsive contractions is a characteristic feature of opioid agonists (Vaughan Williams, 1954; Kosterlitz & Lees, 1964; Burks, Hirning, Galligan & Davis, 1982; Fox & Daniel, 1987).

It has been suggested from studies in the rat colon and guinea-pig small intestine that opioid peptide-containing nerves participate in the peristaltic reflex. Opioid peptides, measured in the superfusate passing over segments of isolated colon (Grider & Makhlouf, 1987*a*, *b*) and in the vascular perfusate of isolated small intestine (Clark & Smith, 1981, 1985; Donnerer *et al.* 1984), decrease during descending relaxation and increase during ascending contraction. In the rat colon, exogenously added [Met^5]enkephalin and dynorphin decrease the release of vasoactive intestinal peptide-like immunoreactive material (Grider & Makhlouf, 1987*a*, *b*). Thus, release of opioid peptides during ascending contraction would be expected to suppress activity in inhibitory motor neurones containing VIP. Although the present studies provide no information on the identity of the inhibitory motor neurone innervating circular muscle of the canine duodenum, they do provide direct electrophysiological evidence supporting the hypothesis that opioid peptides decrease release of an inhibitory transmitter(s).

The opioid receptor type(s) mediating suppression of inhibitory transmission was analysed in the present study by examining the effects of opioid agonists with different affinities for δ-, μ- and κ-receptors. U-50,488H, a substance with greater affinity for κ-receptors than for δ- and μ-receptors (Vonvoigtlander *et al.* 1983) was relatively inactive, whereas DPDPE and PL017, agonists with greater affinity for δ- and μ-receptors respectively, depressed inhibitory transmission with the δ-agonist having greater potency. The occurrence of both δ- and μ-receptors on inhibitory motor nerves has been previously suggested (Egan & North, 1981). The lack of evidence for the presence of κ-receptors receives support from previous studies of mechanical activity (Hirning, Porreca & Burks, 1985; Vaught, Cowan & Jacoby, 1985). There is the possibility that a subtype of κ-receptor exists which was not identified either by our or previous studies. Also, κ-receptors may be restricted to excitatory, cholinergic motor nerves. In the canine small intestine *in vivo*, dynorphin (1–13) given intra-arterially acts on κ-receptors to block atropine-sensitive contractions evoked by electrical field stimulation (Fox & Daniel, 1987). The observations made in the present study should also not be taken to exclude opioid modulation through κ-receptors located in other regions of the gastrointestinal tract (Telford, Caudill, Condon & Szurszewski, 1988).

The inhibitory effect of [Leu^5]- and [Met^5]enkephalin on IJP amplitude was very probably mediated by δ-receptors. The receptor type responsible for the inhibitory effect of dynorphin (1–13) is open to interpretation. Although dynorphin (1–13) is an endogenous ligand for κ-receptors (Chavkin *et al.* 1982), it seems unlikely that exogenously added dynorphin (1–13) acted on κ-receptors because as already

mentioned, U-50,488H, a κ-receptor agonist, had no effect on IJP amplitude. One possible explanation is that tissue enkephalinases degraded dynorphin (1–13) to either [Leu^5]enkephalin or dynorphin (1–8) both of which act on δ-receptors (Yoshimura, Hiudobro-Toro, Lee, Loh & Way, 1982).

The mechanism responsible for suppression of inhibitory transmission was not explored in the present study. Previous studies indicate that opioid peptides increase potassium conductance and/or decrease a voltage-dependent calcium conductance (Cherubini & North, 1985; McFadzean, 1988). A similar mechanism of action in the canine duodenum by opioid peptides on inhibitory nerves would be expected to decrease the safety factor for nerve impulse thereby preventing nerve impulses from reaching the secretory terminals.

The electrophysiological characteristics of circular smooth muscle cells of the duodenum remain to be discussed. The resting membrane potential in circular smooth muscle cells located close to the myenteric plexus was similar to that reported for similarly located muscle cells in the canine distal jejunum (Hara *et al.* 1986). Spontaneously occurring phasic contractions of the circular muscle layer were spike independent, and spontaneously occurring slow waves were absent in preparations of circular muscle free of both plexuses. Similar observations have been made in canine jejunum (Hara *et al.* 1986; Hara & Szurszewski, 1986). Thus, circular smooth muscle cells located in the canine jejunum and duodenum appear to exhibit common electrophysiological features.

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