Br. J. Pharmacol. (1988), **95**, 329–334

Effects of naloxone and opioid agonists on gastric excitatory responses to stimulation of the vagus nerve in cats

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1 An investigation was made into the contribution of endogenous opioids to the initial and delayed excitatory response of the stomach induced by stimulation of the vagal trunk in cats.

2 Naloxone (100 to 1000 $\mu g\,kg^{-1}$) had no effect on the initial excitatory response to stimulation of the vagal efferent fibres. However, the same treatment dose-dependently enhanced the delayed excitatory response to stimulation of the vagal afferent fibres.

3 In comparison with the μ-opioid-receptor agonist, [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin and the κ-opioid-receptor agonist dynorphin A (1–13), lower doses of methionine enkephalin ([Met]enkephalin) markedly inhibited the excitation caused by stimulation of the vagal efferent and afferent fibres. The inhibitory effect of [Met]enkephalin was antagonized by naloxone.

4 The δ-opioid-receptor selective agonist [D-Pen^2, D-Pen^5]enkephalin mimicked the inhibitory effects of [Met]enkephalin and inhibition by [D-Pen^2, D-Pen^5]enkephalin was antagonized by the δ-opioid-receptor antagonist, ICI 174,864.

5 It is concluded that the inhibitory effects of exogenous opioids on the excitatory response of the stomach to stimulation of the vagal efferent and afferent fibres are mediated, at least in part, by δ-opioid-receptors. Naturally occurring opioids may participate in the inhibition of the delayed gastric excitation to stimulation of the vagal afferent fibres.

Introduction

Recently, it was reported that electrical stimulation of the vagal trunk produced a two-phase excitatory response of the stomach in cats: an initial excitatory response during stimulation and a delayed excitatory response after the stimulation period. These responses seemed to be mediated through the efferent and afferent fibres, respectively (Kurahashi *et al.*, 1983; Okamoto *et al.*, 1986).

Narcotic analgesic drugs, such as morphine, have a marked effect on gastrointestinal function. This effect has long been exploited both therapeutically and experimentally. Morphine was shown to cause relaxation of the guinea-pig intestine (Weinstock, 1971) and to inhibit the peristaltic reflex of the guinea-pig isolated ileum by reducing the output of the acetylcholine from the nerves in the myenteric plexuses which innervate the muscle layers (see, Cherubini *et al.*, 1985). Opioid-like materials, behaving like enkephalin (Schulz *et al.*, 1977) and endorphin (Puig *et al.*, 1976), were found to be released from the myenteric plexus-longitudinal muscle preparations of guinea-pigs. These facts suggest the possibility that in these animals, naturally occurring opioids contribute to the regulation of intestinal motility.

Daniel (1966) showed that morphine inhibited gastric emptying in the dog and he further suggested that inhibition of gastric peristalsis either in duodenal spasm or in antral spasm could account for delayed gastric emptying. We found that morphine had no effect on the initial excitatory response of the stomach, but this agent dose-dependently inhibited the delayed excitatory response (Okamoto *et al.*, 1986).

The purpose of these experiments was to explore the contribution of endogenous opioid to the excitatory response of the stomach to stimulation of the vagal efferent and afferent fibres in cats.

Methods

Thirty-five cats of either sex, weighing 2.5 to 4.0 kg were used. The animals were deprived of food but allowed free access to water 12 h before the experiments. After initial anaesthesia with ether, sodium pentobarbitone (10 mg kg^{-1}, i.v.) was administered. A tracheal cannula was inserted. The right femoral vein was catheterized and gallamine triethiodide (20 mg ml^{-1}) was continuously infused at a constant rate (1.48 ml h^{-1}). Artificial respiration was maintained by a respiration pump. The respiration rate was 15 per min with the air volume at 70 ml per stroke. The left femoral vein was catheterized for drug injection. If the heart rate increased by about 10% or the pupils dilated, additional pentobarbitone (10 mg kg^{-1}) was injected into the left femoral vein; additional anaesthetic was given at least twice during an experiment. The cervical vagal trunks on both sides were cut and the ends were ligated. The distal trunk of the left vagus was placed on a bipolar platinum electrode and covered with cotton wool soaked in liquid paraffin. Propranolol (1 mg kg^{-1}) and phentolamine (2 mg kg^{-1}) pretreatment was used to inhibit α and β-adrenoceptors. Gastric motility was recorded with a balloon introduced via the oesophagus. The system was filled with water and connected to a pressure transducer, thus recording changes in intragastric pressure. The level of intragastric pressure was set at 10 to 15 cmH_2O. Changes in intragastric pressure were then recorded on a polygraph (San-ei Instrument, Tokyo, Japan) through a pressure transducer. A stimulator giving square wave pulses was used (10 Hz in frequency, 3 ms in duration and 10 V in intensity for 10 s).

The following drugs were used: naloxone, gallamine triethiodide (Sigma Chemical Co., St Louis, MO); methionine enkephalin ([Met]enkephalin), dynorphin A (1–13) (Protein Research Foundation, Osaka, Japan); [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAGOL), [D-Pen^2, D-Pen^5]enkephalin (DPDPE) (Peninsula Laboratories); ICI 174,864 (allyl 2-Try-Aib-Phe-Leu-OH, Cambridge Research Biochemicals) and ether (Nakarai Chemical Co., Kyoto, Japan).

The height of the excitatory response of the stomach before administration of drug was regarded as 100%.

Statistical analysis was performed by use of Student's t test for unpaired data.

Results

Effects of naloxone

The effects of naloxone on the initial and the delayed excitatory responses of the stomach to stimulation of the vagal trunk were studied in 7 cats. Naloxone was administered 1 min before the stimulation. Naloxone (100 to 1000 $\mu g\,kg^{-1}$, i.v.) had no effect on the initial excitatory response. The same treatment augmented the delayed excitatory response dose-dependently (Figures 1 and 2), to about 180% of the control response. Excitatory responses returned to the control level after 20 to 30 min. Basal tone was not affected by naloxone.

Effects of [Met]enkephalin, dynorphin A (1-13) and DAGOL

The effects of [Met]enkephalin, dynorphin A (1-13) and DAGOL were examined in 13 cats. Each drug

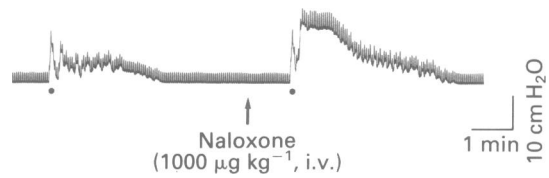

Figure 1 Effects of naloxone (1000 $\mu g\,kg^{-1}$, i.v.) on the initial and the delayed excitatory response of the stomach to electrical stimulation of the vagal trunk in cats. Vertical scale indicates 10 cmH_2O; horizontal scale 1 min; (●) the initial excitatory response.

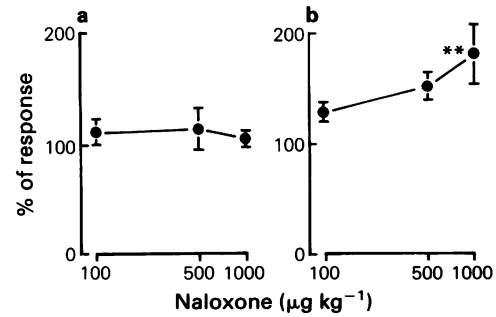

Figure 2 Effects of naloxone (100 to 1000 $\mu g\,kg^{-1}$, i.v.) on the initial (a) and the delayed (b) excitatory response of the stomach to electrical stimulation of the vagal trunk in cats. Ordinate scale: % response as compared with control response before administration of naloxone. Abscissa scale: dose of naloxone. ** Statistically significant difference from corresponding control, by Student's t test ($P < 0.05$).

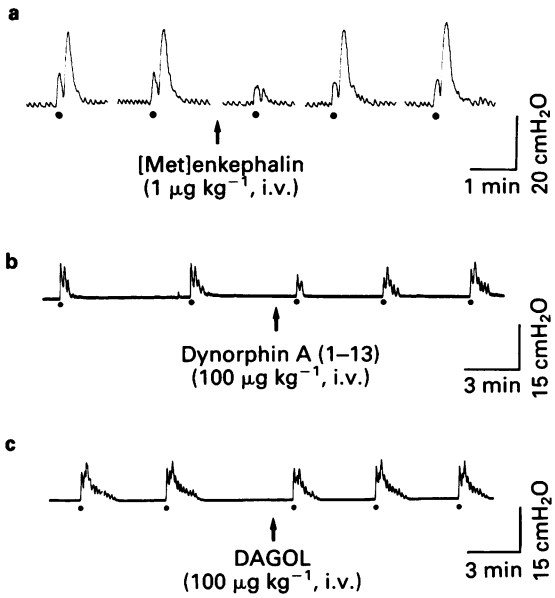

Figure 3 Effects of [Met]enkephalin, dynorphin A (1-13) and [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAGOL) on the initial and the delayed excitatory response of the stomach to electrical stimulation of the vagal trunk in cats. (a) Effects of [Met]enkephalin (1 $\mu g\,kg^{-1}$, i.v.). Vertical scale indicates 20 cmH_2O, horizontal scale 1 min; (●) initial excitatory response. (b) Effects of dynorphin A (1-13) (100 $\mu g\,kg^{-1}$, i.v.). Vertical scale indicates 15 cmH_2O, horizontal scale 3 min; (●) initial excitatory response. (c) The same as in (b) except DAGOL (100 $\mu g\,kg^{-1}$, i.v.) was used instead of dynorphin A (1-13).

was administered 1 min before stimulation. The results were as follows; in 7 cats, the initial and the delayed excitatory responses were inhibited dose-dependently within 3 min after injection of [Met]enkephalin (0.1 to 10 $\mu g\,kg^{-1}$) (Figures 3a and 4A). Basal tone was not affected by [Met]enkephalin. Such inhibition did not continue for more than 7 min. Judging from the ID_{50} for inhibition of the initial and the delayed excitatory responses, [Met]enkephalin was about 6 times more effective on the delayed excitation than on the initial excitation (Table 1). There was no significant difference between the effects of [Met]enkephalin and [Leu]enkephalin (not shown). In 3 cats, dynorphin A (1-13) (10 to 100 $\mu g\,kg^{-1}$) did not affect basal tone and caused only weak inhibition on both the initial and the delayed excitatory responses (Figures 3b and 4B). In these cases, the inhibitory effect of dynorphin A (1-13) (10 to 100 $\mu g\,kg^{-1}$) was less powerful than that of [Met]enkephalin (Table 1). Finally, in 3 cats, DAGOL (10 to 100 $\mu g\,kg^{-1}$) did not affect basal

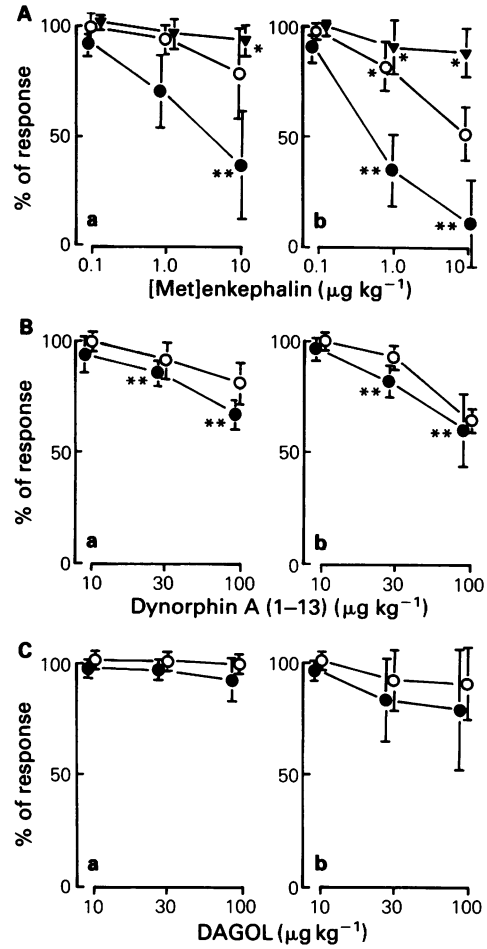

Figure 4 Effects of [Met]enkephalin, dynorphin A (1-13) and [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAGOL) on the initial (a) and the delayed (b) excitatory response of the stomach to electrical stimulation of the vagal trunk in cats. (A) Effects of [Met]enkephalin (0.1 to 10 $\mu g\,kg^{-1}$, i.v.). Ordinate scale: % response as compared with control response before administration of [Met]enkephalin. Abscissa scale: dose of [Met]enkephalin. (●) Control, (○) pretreatment with naloxone (100 $\mu g\,kg^{-1}$, i.v.) and (▼) pretreatment with naloxone (1000 $\mu g\,kg^{-1}$, i.v.). ** Statistically significant difference from corresponding control ($P < 0.05$). * Statistically significant difference from corresponding [Met]enkephalin-induced inhibition, by Student's t test ($P < 0.05$). (B) Effects of dynorphin A (1-13) (10 to 100 $\mu g\,kg^{-1}$, i.v.). Ordinate scale: % response as compared with control response before administration of dynorphin A (1-13). (●) Control, (○) pretreatment with naloxone (500 $\mu g\,kg^{-1}$, i.v.). ** Statistically significant difference from corresponding control, by Student's t test ($P < 0.05$). (C) The same as in (B) except that DAGOL was used instead of dynorphin A (1-13).

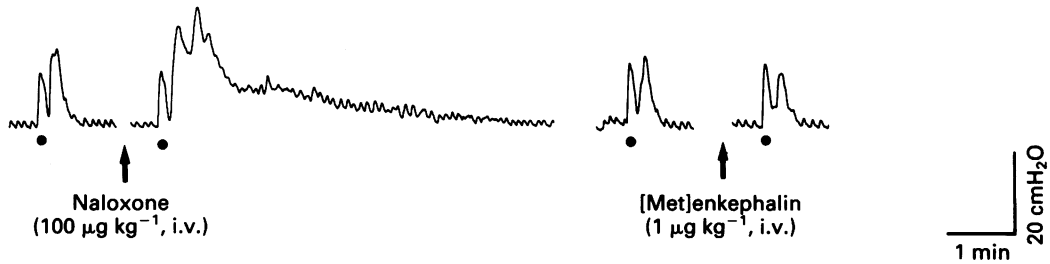

Figure 5 Effects of naloxone (100 $\mu g\,kg^{-1}$, i.v.) on the [Met]enkephalin (1 $\mu g\,kg^{-1}$, i.v.)-induced inhibition of the initial and the delayed excitatory response of the stomach to electrical stimulation of the vagal trunk in cats. Vertical scale indicates 20 cmH_2O; horizontal scale 1 min; (●) initial excitatory response.

Table 1 ID_{50} of [Met]enkephalin, dynorphin A (1-13), [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAGOL) and [D-Pen^2, D-Pen^5]enkephalin (DPDPE) on the initial and the delayed excitatory response of the stomach to stimulation of vagal trunk

	ID_{50} ($\mu g\,kg^{-1}$)	
Agonist	*Initial excitation*	*Delayed excitation*
[Met]enkephalin	7.6 ± 7.1	1.3 ± 1.1
Dynorphin A (1-13)	> 100	> 100
DAGOL	> 500	> 500
DPDPE	1.0 ± 0.2	1.0 ± 0.1

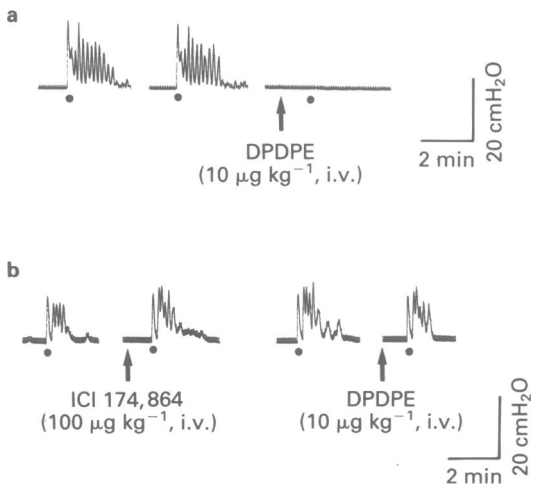

Figure 6 Effects of [D-Pen^2, D-Pen^5]enkephalin (DPDPE) on the initial and the delayed excitatory response of the stomach to electrical stimulation of the vagal trunk in cats. Vertical scale indicates 20 cmH_2O; horizontal scale 2 min; (●) initial excitatory response. (a) DPDPE (10 $\mu g\,kg^{-1}$, i.v.); (b) DPDPE (10 $\mu g\,kg^{-1}$, i.v.) following pretreatment with ICI 174,864 (100 $\mu g\,kg^{-1}$, i.v.).

tone and caused no significant inhibition on either the initial or the delayed excitatory response (Figures 3c and 4C).

Effects of naloxone on [Met]enkephalin-, dynorphin A (1-13)- and DAGOL-induced inhibition

[Met]enkephalin has been shown to inhibit the initial and the delayed excitatory response. On the other hand, dynorphin A (1-13) and DAGOL have shown weak inhibition on the excitatory responses. A further set of experiments was conducted on 9 cats to test the effects of naloxone on the inhibitory effects. The results of these experiments were as follows: in 3 cats, the augmentation of gastric response due to naloxone (100 and 1000 $\mu g\,kg^{-1}$) was allowed to return to the control level. [Met]enkephalin (0.1 to 10 $\mu g\,kg^{-1}$) was then administered intravenously, 1 min before stimulation. Pretreatment with naloxone attenuated the inhibitory effects of [Met]enkephalin on both the initial and the delayed excitatory response of the stomach (Figures 4A and 5). In another 3 cats pretreated with naloxone (500 $\mu g\,kg^{-1}$), dynorphin A (1-13) (10 to 100 $\mu g\,kg^{-1}$) was administered intravenously, 1 min before stimulation. The pretreatment with naloxone did not attenuate the inhibitory effects of dynorphin A (1-13) significantly (Figure 4B). In the last 3 cats, naloxone did not affect the inhibitory effects of DAGOL (Figure 4C).

Effects of DPDPE and ICI 174,864

The effects of DPDPE and ICI 174,864 on the initial and the delayed excitatory response of the stomach to stimulation of vagal trunk were studied in 6 cats. In 3 cats, DPDPE was administered 1 min before the stimulation. Basal tone was not affected by DPDPE. DPDPE (0.1 to 10 $\mu g\,kg^{-1}$) inhibited both the initial and the delayed excitatory responses dose-dependently (Figures 6a and 7). Judging from the

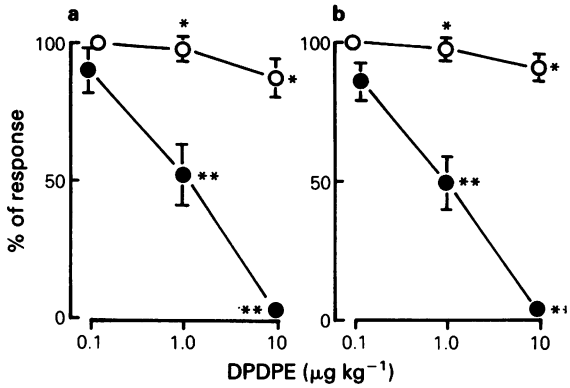

Figure 7 Effects of [D-Pen^2, D-Pen^5]enkephalin (DPDPE, 0.1 to 10 $\mu g\,kg^{-1}$, i.v.) on the initial (a) and the delayed (b) excitatory response of the stomach to electrical stimulation of the vagal trunk in cats. Ordinate scale: % response as compared with control response before administration of DPDPE. Abscissa scale: dose of DPDPE. (●) Control; (○) pretreatment with ICI 174,864 (100 $\mu g\,kg^{-1}$, i.v.). ** Statistically significant difference from corresponding control ($P < 0.01$). * Statistically significant difference from corresponding DPDPE-induced inhibition by Student's t test ($P < 0.01$).

ID_{50} for inhibition of the response, DPDPE has almost the same potency as [Met]enkephalin in inhibiting the delayed excitatory response (Table 1). In another 3 cats, ICI 174,864 (100 $\mu g\,kg^{-1}$) was administered about 15 min before DPDPE. ICI 174,864 did not affect the basal tone but augmented the delayed excitatory response to about 35% of the control response. ICI 174,864 antagonized the inhibitory effects of DPDPE (Figures 6b and 7).

Discussion

We have shown that two types of gastric excitatory responses are produced by stimulation of the vagal trunk in cats. The initial excitatory response is inhibited by hexamethonium and atropine; the delayed excitatory response is not inhibited by hexamethonium, but is inhibited by atropine. In chronically supranodose vagotomized cats, only a delayed excitatory response was observed. Thus, it was considered that the initial excitatory response and the delayed excitatory response were induced through stimulation of the efferent and afferent fibres, respectively (Kurahashi *et al.*, 1983; Okamoto *et al.*, 1986).

In the present experiments, naloxone enhanced the hexamethonium-resistant delayed excitatory response of the stomach. This effect of naloxone may have resulted from the blockade of the action of endogenously occurring opioids. It was reported (Edin, 1980; Edin *et al.*, 1980) that the gastric contraction induced by stimulation of the vagal efferent fibres with pulses of 5 ms duration was converted to relaxation by the administration of hexamethonium or atropine and that such relaxation was reduced by naloxone. The administration of enkephalin before treatment with hexamethonium or atropine, caused relaxation of the stomach and this effect was also reduced by naloxone. Thus, endogenous opioids may have contributed to gastric relaxation caused by stimulation of the vagal efferent fibres. However, Delbro *et al.* (1981) and Tsubomura *et al.* (1987) considered that vagal stimulation with pulses of such long duration may have activated not only efferent but also afferent fibres. Delbro *et al.* (1982) reported that the hexamethonium-resistant gastric motor response in cats, may be due to antidromic activation of the vagal afferent fibres. Unlike our results however, this response was not affected by naloxone.

Morphine is known to inhibit gastric emptying in the dog and information as to the site of its action is available (Daniel, 1966). The latter author argued that inhibition of gastric peristalsis either in duodenal spasm or in antral spasm could account for the delayed gastric emptying. In previous experiments (Okamoto *et al.*, 1986), we found that morphine had no effect on the initial excitatory response but inhibited the delayed excitatory response of the stomach. Although morphine is a prototype μ-opioid-receptor agonist, it has been shown to interact with δ- and κ-opioid-receptors (Paterson *et al.*, 1983). Thus, it is not possible to define the receptor type on which morphine acts in this case. In this study, [Met]enkephalin (a δ-opioid-receptor agonist) in low doses inhibited the initial and the delayed excitatory responses dose-dependently, and these effects were antagonized by naloxone. DAGOL (μ-opioid-receptor agonist) did not significantly inhibit either the initial or the delayed excitatory response. High doses of dynorphin A (1-13) (κ-opioid-receptor agonist) exhibited weak inhibition of both responses; however, this inhibitory effect of dynorphin A (1-13) was not antagonized by naloxone. Furthermore, low doses of the δ-opioid-receptor selective agonist, DPDPE (Mosberg *et al.*, 1983), mimicked the inhibitory effects of [Met]enkephalin and the inhibition by DPDPE was antagonized by the δ-opioid-receptor selective antagonist ICI 174,864 (Cotton *et al.*, 1984). The results indicate that the inhibitory effects of opioids were mediated, at least in part, by δ-opioid-receptors. Immunofluorescence studies have already demonstrated the presence of enkephalin-like immunoreactive materials in the vagus nerves (Elde *et al.*, 1976; Lundberg *et al.*, 1978; Lundberg *et al.*, 1979)

and stomach (Polak *et al.*, 1977). Our results indicate that [Met]enkephalin is the most probable endogenous opioid to inhibit the delayed excitatory response of the stomach to stimulation of afferent vagal fibres. This implies the possibility that an endogenous enkephalin-like opioid may contribute to the inhibitory modulation of gastric motility.

This study was supported in part by a Grant-in-Aid from the Smoking Research Foundation, Japan.

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(Received August 4, 1987
Revised May 4, 1988
Accepted May 17, 1988)