Opposite central and peripheral control by endogenous opioids of intestinal motility in fed rats

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1 The effects of the inhibitors of endopeptidase EC 24.11, thiorphan and phosphoramidon administered i.c.v. ($40 \mu\text{g kg}^{-1}$) i.p. ($400 \mu\text{g kg}^{-1}$), or orally ($400 \mu\text{g kg}^{-1}$), on intestinal motor activity in fed rats was compared to the effects of similar doses of the angiotensin converting enzyme inhibitor, captopril and the synthetic enkephalin analogue [D-Ala² Met⁵] enkephalinamide (Dalamide). Drugs were administered alone or after pretreatment with naloxone or N-methyl levallorphan ($300 \mu\text{g kg}^{-1}$, i.p.) given 10 min prior to gavage with a standard meal.

2 In control conditions, in the duodenum, the disruption of the migrating myoelectric complex (MMC) by gavage with a standard meal lasted between 105.6 and 119.1 min. This duration was significantly decreased by thiorphan (60.3 ± 15.0 min), phosphoramidon (67.9 ± 7.3 min), captopril (26.3 ± 10.2 min) and Dalamide (42.4 ± 9.6 min), administered i.c.v.

3 In contrast, after the i.p. administration of thiorphan, phosphoramidon and Dalamide the delay in the return of the MMC pattern was increased. Such an effect was also seen after the oral administration of phosphoramidon or Dalamide. Neither i.p. nor oral captopril administration altered the duration of postprandial pattern.

4 A prior treatment with naloxone i.p. ($300 \mu\text{g kg}^{-1}$) that had no effect *per se*, antagonized the effect produced by i.c.v. administration of thiorphan, phosphoramidon or Dalamide, but failed to reverse the effect of captopril. In contrast, i.p. administration of N-methyl levallorphan ($300 \mu\text{g kg}^{-1}$) did not affect the response induced by central administration of thiorphan, phosphoramidon, captopril or Dalamide, but was able to prevent that of thiorphan, phosphoramidon or Dalamide when they were administered i.p. or orally.

5 These data strongly support the hypothesis of a dual control by endogenous opioids of intestinal motility in the rat: a central component that favours, and a peripheral control that delays the occurrence of the MMC profile in fed rats.

Introduction

Various preparations and extracts of the opium poppy were in use for their antidiarrhoeal effects some time before the exploitation of the plant for its analgesic properties (see Mannara & Bianchetti, 1985). The pioneering work by Margolin (1954) suggested that this constipating effect could be related to a slowing of intestinal transit in mice. More recently, it was shown that morphine also delays gastric emptying in rats (Stewart & Curd, 1984), and that this antipropulsive action on the gastrointestinal tract is associated with a decrease in contractile activity (Weisbrodt *et al.*, 1980; Galligan & Burks, 1983). Furthermore, because these effects occur after direct administration of small amounts to

the brain as well as by administration via a peripheral route, a central origin for the action of morphine on the gut has been suggested (Buéno & Ruckebusch, 1978; Stewart *et al.*, 1979). The many inconsistencies that appear in the literature concerning the possible site of action of opioids in affecting gut motility can be explained on the basis of differences in experimental conditions, routes of administration, opioid agonist used, species or gastrointestinal segment considered (see for reviews: Mannara & Bianchetti, 1985; Buéno & Fioramonti, 1988). Numerous works (Galambos *et al.*, 1976; Schultz *et al.*, 1979; Buéno *et al.*, 1985; Fioramonti *et al.*, 1987) suggest a possible direct action of opiates on the gut which is in agreement with the previous identification of opioid receptors (Kosterlitz *et al.*, 1972) and

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enkephalins (Polak *et al.*, 1977) in the digestive tract. Despite numerous studies using exogenous administrations of stable long acting analogues of enkephalins, only a few studies have addressed the possible physiological participation of endogenous opioid peptides in the long term regulation of intestinal functions.

An understanding of the enzymatic breakdown of the enkephalins has led to the synthesis of potent inhibitors of 'enkephalinase', endopeptidase EC 24.11. Such an inhibitor, thiorphan (Roques *et al.*, 1980), does not cross the blood brain barrier (BBB) (Schwartz *et al.*, 1985), and thus allows the possibility of preventing selectively the degradation of endogenous opioid peptides at central or peripheral sites. Using thiorphan or its precursor (acetorphan), the possible involvement of a central control by enkephalins of intestinal water and ion transport has been demonstrated in the dog (Primi, 1986). Likewise, the existence of both a central inhibitory and a peripheral stimulatory control by endogenous opioids of gastric emptying of a nutritive fat meal in the mouse has recently been proposed (Liberge *et al.*, 1988).

The present study was performed in fed rats to investigate the site of action of endogenous opioid peptides that may be involved in the control of intestinal motility by use of a chronic electromyographic technique. The effects on intestinal motor activity in fed rats of intracerebroventricular (i.c.v.), intraperitoneal (i.p.), and oral (p.o.) administration of thiorphan have been compared with those of similar treatments by phosphoramidon, another inhibitor of endopeptidase EC 24.11 (Schwartz *et al.*, 1985), by captopril, selective inhibitor of angiotensin converting enzyme (ACE) (Schwartz *et al.*, 1981) and by [D-Ala²-Met⁵] enkephalinamide (Dala-mide), a long acting synthetic analogue of enkephalins. These drugs were administered either alone or following a prior treatment with the opioid antagonists naloxone or N-methyl levallorphan.

Methods

Animal preparation

Twelve male Wistar rats weighing 300–400 g, housed individually and maintained at an ambient temperature of 20–21°C, were used in the study. Under halothane anaesthesia, animals were prepared for a long term electromyographic recording of intestinal motility (Ruckebusch & Fioramonti, 1975) by implantation in the intestinal wall of 4 groups of 3 nichrome wires (80 µm in diameter) placed on the small intestine at 5, 10, 20 and 25 cm from the pylorus. The electrode wires (60 cm in length) were

exteriorized on the back of the neck. In addition, a small polyethylene catheter was inserted into each lateral ventricle of the brain (Stewart *et al.*, 1978).

Recordings

Recordings were started 5 days after surgery. The electrical activity of the intestine was recorded each day from 09 h 00 min to 19 h 00 min with an electroencephalograph (Reega VIII, Alvar, Paris) having a paper speed of 2.4 cm min⁻¹. The identification of the intestinal motor pattern was facilitated by the summation of the spiking activity every 20 s; this gave an integrated record of the myoelectrical activity which was plotted continuously through each 24 h period on the y axis of a potentiometric recorder with a paper speed of 5 cm h⁻¹ (Latour, 1973).

Experimental procedure

The experiments were performed each day at 13 h 00 min, following 5 h of food deprivation.

In a first series of experiments, the rats received either 5 µl (i.c.v.), 0.2 ml (i.p.) or 0.2 ml (p.o.) of sterile isotonic saline alone (vehicle) or containing thiorphan (Laboratoire Bioprojet, Paris), phosphoramidon (Sigma, France), captopril (Squibb, Paris) or Dala-mide (Sigma, France) at doses of 40 µg kg⁻¹, i.c.v., and 400 µg kg⁻¹ i.p. or p.o. Either treatment was followed 10 min later by gavage with a 3 ml nutritive meal of hydrated cow's milk powder (sugars 0.5 g; fats 0.5 g; proteins 0.5 g).

In a second series of experiments, the same experimental model and procedure were used to compare the effects of two opiate antagonists: naloxone (Endo laboratories, Garden City, New York) that is active at central and peripheral sites after parenteral administration, and a quaternary narcotic antagonist, N-methyl levallorphan (SR 58002 C, Sanofi-Recherche, Montpellier, France) that penetrates to the brain poorly (Bianchetti *et al.*, 1985). Each of these was administered at different doses i.p. (50 to 2000 µg kg⁻¹) in order to determine the threshold for alteration of postprandial motility.

In a third series of experiments, the highest dose of naloxone or of N-methyl levallorphan that had no effect by itself (300 µg kg⁻¹, i.p.) was administered 10 min prior to the i.c.v., i.p. or p.o. administration of thiorphan, phosphoramidon, captopril, and Dala-mide.

The effect of the different treatments was evaluated by measuring the duration of the disruption of the postprandial MMC in the duodenum and the jejunum. At the end of the experiment, after the return of the fasted motility pattern, the animals were fed with 8 g of pelleted laboratory chow for

rodents (Usine d'Alimentation Rationnelle, Epinay, France).

Statistical analysis

Data were analysed using the non-parametric Wilcoxon-test that allows paired comparisons and are given as mean $\pm$ s.d.

Results

The continuous integrated (summation of the spikes every 20 s) recording of small intestinal myoelectrical activity in conscious rats shows (Figure 1) two distinct intestinal motor activity profiles (Ruckebusch & Fioramonti, 1975). In fasted animals, the motility is characterized by the occurrence of a Migrating Myoelectrical Complex (MMC) the mean duration of which is 12 to 18 min and which migrates at a speed of 2 to 3 cm per min. The MMC is organised in three different phases: a period of quiescence

(phase I) followed sequentially by periods of irregular (ISA or phase II) and regular spiking activity (RSA or phase III). After feeding, this profile is replaced by a continuous ISA.

Experiment 1

The gavage with a standard meal was followed by a disruption of the MMC pattern in both duodenum and jejunum, which was not affected by the administration of the vehicle, irrespective of the route used (i.c.v., i.v. or i.p.). The duration of the postprandial pattern was significantly decreased by the i.c.v. administration of thiopran, phosphoramidon (Figure 1), captopril or Dalamide (respectively 60.3 ± 15.0 min; 67.9 ± 7.3 min; 26.3 ± 10.2 min and 42.4 ± 9.6 min in the duodenum, $n = 12$, Figure 2). The opposite effects were observed after i.p. administration of thiopran, phosphoramidon or Dalamide where the recovery of the MMC pattern was delayed and the period during which ISA was found was increased by at least 18%. This last effect was reproduced by oral administration of phosphoramidon or

Figure 1 Effects of i.c.v. ($40 \mu\text{g kg}^{-1}$), i.p. ($400 \mu\text{g kg}^{-1}$) and p.o. ($400 \mu\text{g kg}^{-1}$) administration of phosphoramidon on duodenal myoelectrical activity (integrated record) of rats fed by gavage with a test meal; i.c.v. treatment shortens while i.p. and p.o. administration delay the return of the fasted pattern (MMC). μC = microcoulomb.

Figure 2 Effects of i.c.v. ($40 \mu\text{g kg}^{-1}$), i.p. ($400 \mu\text{g kg}^{-1}$) and p.o. ($400 \mu\text{g kg}^{-1}$) administration of thiorphan, phosphoramidon, captopril and Dalamide on the duration of the postprandial pattern on duodenum following gavage with a test meal in rats; columns show mean values ($n = 12$) and vertical bars indicate s.d. Open columns, vehicle; vertically lined columns, thiorphan; stippled columns, phosphoramidon; solid columns, captopril; hatched columns, Dalamide. * $P < 0.05$, W-test.

Dalamide but not by thiorphan which induced an early return of the MMC profile. Neither the i.p. nor p.o. administration of captopril altered the duration of the postprandial pattern (Table 1).

Figure 3 Effects of i.p. administration of naloxone (1 mg kg^{-1}) and N-methyl levallorphan (2 mg kg^{-1}) on the duodenal fed pattern following gavage with a test meal in rats; columns show mean values ($n = 12$) and vertical bars indicate s.d. Open column, vehicle; hatched column, naloxone; solid column, N-methyl levallorphan.

Experiment 2

Intraperitoneal administration of naloxone (1 mg kg^{-1}) and N-methyl levallorphan (2 mg kg^{-1}) alone produced opposite effects, increasing (+19%) and decreasing (−12%) respectively the duration of the postprandial pattern at both duodenal (Figure 3) and jejunal levels. However, they failed to alter the pattern when given at a lower dose ($300 \mu\text{g kg}^{-1}$, i.p.).

Experiment 3

Previous i.p. treatment by a lower dose of naloxone of $300 \mu\text{g kg}^{-1}$ that had no effect by itself antagonized the effect of i.c.v. administration of thiorphan, phosphoramidon or Dalamide, but failed to reverse that of captopril (Table 2). In contrast, N-methyl levallorphan ($300 \mu\text{g kg}^{-1}$) did not affect significantly ($P \geq 0.05$) the response to i.c.v. administration of thiorphan, phosphoramidon, captopril or Dalamide but suppressed the action of thiorphan, phos-

Table 1 Action of i.c.v., i.p. and p.o. administration of thiorphan, phosphoramidon, captopril and Dalamide on the inhibition of the jejunal migrating myoelectric complex (MMC) pattern observed following gavage with a test meal in rats

	Postprandial disruption of the jejunal MMC pattern (min)				
	Vehicle	Thiorphan	Phosphoramidon	Dalamide	Captopril
i.c.v.	94.3 ± 10.7	$55.6 \pm 15.6^*$	$60.7 \pm 7.9^*$	$39.3 \pm 8.2^*$	$20.1 \pm 9.1^*$
i.p.	102.4 ± 15.9	$123.8 \pm 14.0^*$	$125.7 \pm 12.2^*$	$128.5 \pm 23.0^*$	95.3 ± 15.0
p.o.	105.1 ± 17.1	$71.5 \pm 24.2^*$	$182.3 \pm 26.5^*$	$170.8 \pm 20.6^*$	100.5 ± 23.4

* Significantly different from vehicle values (W-test, $P \leq 0.05$, $n = 12$).

Table 2 Influence of previous i.p. ($300 \mu\text{g kg}^{-1}$) administration of naloxone or N-methyl levallorphan (NML) on the effects of i.c.v., i.p. and p.o. administration of thiorphan, phosphoramidon, captopril and Dalamide on the inhibition of the duodenal migrating myoelectric complex (MMC) pattern induced by gavage with a test meal in rats

	Route	Postprandial disruption of the duodenal MMC pattern (min)		
		Control	Naloxone, i.p.	NML, i.p.
Vehicle	i.c.v.	105.6 $\pm$ 11.8	110.2 $\pm$ 16.4	113.1 $\pm$ 18.8
	i.p.	111.4 $\pm$ 17.8	106.8 $\pm$ 9.8	118.1 $\pm$ 17.4
	p.o.	119.1 $\pm$ 19.0	107.3 $\pm$ 14.5	116.5 $\pm$ 11.2
Thiorphan	i.c.v.	60.3 $\pm$ 15.0*	107.0 $\pm$ 10.8†	71.4 $\pm$ 17.0*
	i.p.	131.8 $\pm$ 15.3*	117.2 $\pm$ 15.1†	109.2 $\pm$ 10.7†
	p.o.	84.8 $\pm$ 28.0*	112.4 $\pm$ 13.5†	104.0 $\pm$ 13.0†
Phosphoramidon	i.c.v.	67.9 $\pm$ 7.3*	112.9 $\pm$ 13.7†	58.1 $\pm$ 12.5*
	i.p.	132.8 $\pm$ 14.0*	102.7 $\pm$ 10.3†	112.3 $\pm$ 14.4†
	p.o.	194.1 $\pm$ 34.1*	109.0 $\pm$ 12.6†	103.7 $\pm$ 12.1†
Dalamide	i.c.v.	42.4 $\pm$ 9.6*	117.3 $\pm$ 16.7†	49.3 $\pm$ 7.0*
	i.p.	142.6 $\pm$ 22.9*	103.5 $\pm$ 12.1†	111.6 $\pm$ 15.2†
	p.o.	182.4 $\pm$ 22.0*	106.1 $\pm$ 17.7†	100.7 $\pm$ 9.4†
Captopril	i.c.v.	26.3 $\pm$ 10.2*	30.1 $\pm$ 8.5*	24.9 $\pm$ 11.2*

* Significantly different from vehicle values; † significantly different from control values (W-test, $P \leq 0.05$, $n = 12$).

phoramidon or Dalamide injected i.p. or administered orally.

Discussion

Previous studies have shown that inhibitors of endopeptidase EC 24.11 affect intestinal absorption processes in the dog (Primi, 1986) and gastric emptying in mice (Liberge *et al.*, 1988), and the present study demonstrates that the administration of thiorphan alters small intestinal motility in fed rats. The ability of Dalamide or phosphoramidon to reproduce the effects of thiorphan by central or peripheral administration strongly supports the idea that such effects are mediated by endogenous opioid peptides.

Nevertheless, it has been reported that endopeptidase EC 24.11 may be involved in the enzymatic breakdown of other neuropeptides that are involved in the control of small intestinal motility at central and/or peripheral sites. The central administration of thiorphan potentiates the hypothermic effect of neurotensin in mice (Coquerel *et al.*, 1986) and the i.c.v. administration of neurotensin restores a fasted pattern of intestinal motility in fed rats (Buéno *et al.*, 1983), suggesting that neurotensin could be involved in the central action of thiorphan. However, while the potentiation of neurotensin-induced hypothermia by thiorphan is not reversed by naloxone (Coquerel *et al.*, 1986), in our study naloxone is found to be able to abolish the effects of thiorphan on gut motility. Endopeptidase EC 24.11 may also be involved in the degradation of cholecystokinin (CCK)

(Deschodt-Lanckman & Strosberg, 1983), a neuropeptide that is reported to increase the period of the postprandial MMC disruption by a central action (Duc, 1988). However, the latter effect is the opposite of that of thiorphan given i.c.v., suggesting that the central action of thiorphan is not mediated by CCK. Similarly, endopeptidase EC 24.11 may be the major degrading enzyme for atrial natriuretic factor (ANF) (Sonnenberg *et al.*, 1988). Although ANF has a relaxant action on the vascular smooth muscle (Garcia *et al.*, 1985), there is no evidence for an action of ANF on intestinal smooth muscle. Finally the lack of blockade by naloxone of the central effects of captopril on gut motility suggests the possible involvement of a non-opioid mechanism, despite the possibility of breakdown of enkephalins *in vitro* by ACE (Schwartz *et al.*, 1981).

The poor ability of thiorphan (Schwartz *et al.*, 1985) and Dalamide (Rapoport *et al.*, 1980) to cross the BBB suggests that they are acting at the sites of their administration. This last finding is confirmed by the fact that a previous i.p. treatment with naloxone blocks the effects of i.c.v. administration of thiorphan and Dalamide, while N-methyl levallorphan, which does not reach the CNS, is ineffective. In contrast, both antagonists block the effects of the i.p. administration of thiorphan or Dalamide. These data also suggest that phosphoramidon does not penetrate to the CNS to any significant degree.

Our experiments suggest the existence of an opposite central and peripheral control by endogenous opioid peptides of small intestinal motility in fed rats. This observation and its possible physiological relevance are reinforced by the opposite effects

that are produced by the i.p. administration of high doses of naloxone or N-methyl levallorphan given alone. At a central level, enkephalins or related peptides may act to shorten the postprandial pattern that is represented by a permanent irregular spiking activity (ISA), and associated with the highest rate of intestinal transit (Buéno *et al.*, 1975). During the postprandial period of prolonged ISA there may be inhibition of both gastric peristalsis and gastric emptying (Thomas *et al.*, 1934) and the present results are consistent with previous experiments suggesting an inhibition of gastric emptying in mice by a central opioid mechanism (Liberge *et al.*, 1988) and with the central antipropulsive effects of morphine in rats (Galligan & Burks, 1983; Stewart & Curd, 1984). Conversely, by a direct action at a peripheral level, endogenous opioid peptides appear to act to

delay the occurrence of the MMC which is in agreement with the peripheral stimulation of gastric emptying by enkephalins that was observed in mice (Liberge *et al.*, 1988).

In conclusion, using inhibitors of endopeptidase EC 24.11 we have demonstrated the existence in fed rats of central reorganizing and a peripheral disrupting physiological control by endogenous opioid mechanisms of the MMC pattern.

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