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New models for the evaluation of opioid effects in the guinea-pig ileum

Josef Donnerer & Fred Lembeck

Department of Experimental and Clinical Pharmacology of the University of Graz, Universitätsplatz 4, A-8010 Graz, Austria

1 The pharmacology of morphine and opioid peptides was studied in the guinea-pig ileum by examining their inhibitory effects on propulsive peristaltic activity and on the cooling-induced longitudinal contraction.

2 In these experiments, dose-response curves were recorded. The rank order of potency in inhibiting peristalsis was found to be: dermorphin > FK 33-824 > dynorphin-(1–17) > dynorphin-(1–13) > δ-receptor-peptide > morphine ≫ [Leu] enkephalin, whereas the rank order in inhibiting cooling-induced contractions was found to be: dynorphin-(1–13) ≃ FK 33-824 ≃ dermorphin > δ-receptor peptide > morphine. Naloxone antagonized the maximally effective dose of each of the opioid agents.

3 In view of the differences between the abilities of these opioids to inhibit propulsive peristaltic activity, these models seem to be valuable for the examination of inhibitory opioid effects in the gut.

Introduction

In the gut, the site of action of exogenously applied opioids is on cholinergic (Szerb, 1982; Yau *et al.*, 1982) and non-cholinergic (Barthó *et al.*, 1982a; Kromer & Schmidt, 1982) enteric neurones rather than on muscle cells. The most frequently used experiment for comparison of the potencies of opioids in the guinea-pig ileum is the evaluation of their inhibitory effect on cholinergic contractions in response to electrical field stimulation. We investigated the potency of morphine and opioid peptides on the cholingeric longitudinal contraction of the guinea-pig ileum which is induced by rapid cooling (Holzer & Lembeck, 1979b) and on the propulsive peristaltic activity evoked by raised intraluminal pressure, i.e. cholinergic and non-cholinergic contractions of both the circular and longitudinal muscle layers of the ileum (Holzer & Lembeck, 1979a). The present investigation involved substances that are known to be present in the gut such as [Leu] enkephalin (Hughes *et al.*, 1977; Schultzberg *et al.*, 1980), dynorphin-(1–13) and dynorphin-(1–17) (Tachibana *et al.*, 1982; Vincent *et al.*, 1984; Donnerer *et al.*, 1984) as well as a synthetic δ-receptor peptide (Gacel *et al.*, 1980) and dermorphin (Negri *et al.*, 1981). Each of these substances was compared with the known effects of morphine and the stable [Met] enkephalin analogue FK 33-824. Naloxone was used to characterize the action of opioids.

Methods

Guinea-pigs (300–500 g), fasted overnight, were used. To study peristaltic activity in the vascularly perfused ileum preparation, the arrangement described by Holzer & Lembeck (1979a) was used. An ileal segment with its vascular supply was set up in an organ bath containing Tyrode solution at 37°C. The loop of ileum was perfused with oxygenated Tyrode solution via the mesenteric artery at a constant rate of 1.25 ml min^{-1} by a peristaltic pump. The portal vein was also cannulated to drain the venous outflow. A Mariotte bottle containing Tyrode solution was set to raise the intraluminal pressure at the oral end of the ileal segment from 0 to 2.5 mbar for periods of 150 s in cycles of 500 s. The fluid propelled by the peristaltic waves had to pass an outlet mercury valve set to open at a pressure of 3.5 mbar, and was collected in a vertically positioned glass tube. The aboral intraluminal pressure and the amount of fluid propelled (measured as pressure at the lower end of the collecting tube) were recorded by means of Statham transducers (P23BB) on a Beckmann Dynograph. Drugs were infused in volumes of 15–60 μl min^{-1} via the arterial supply. The solvent for the opioid peptides was saline containing 1 g l^{-1} of gelatine to prevent their adsorption to surfaces; morphine and naloxone were dis-

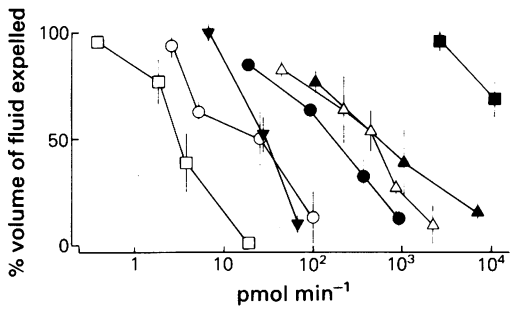

Figure 1 Dose-response relations for morphine and opioid peptides on pressure induced peristalsis of the guinea-pig ileum. Abscissa scale: rate of i.a. infusion. Ordinate scale: volume of fluid expelled in the peristalsis period during infusion of the compounds, expressed as % of the volume expelled in the period immediately prior to drug infusion (100%). Dermorphin (□); dynorphin-(1–13) (●); dynorphin-(1–17) (▼); δ-receptor peptide (△); FK 33-824 (○); morphine (●); [Leu] enkephalin (■). Means of $n = 4–6$; vertical lines show s.e.mean.

solved in saline without gelatine. Drugs were infused during one resting and one peristalsis period; the infusions were started (and stopped) at the beginning of a resting period. Drugs were infused in a randomized order. Each drug infusion was started as soon as peristalsis had returned to normal following administration of the previous compound or after two peristalsis periods following ineffective drug concentrations.

Cooling-induced longitudinal contractions were studied on a segment of ileum suspended in a 6 ml organ bath containing oxygenated Tyrode solution. The segment was kept under a resting load of 0.5 g and connected to an isotonic lever displacement measuring system (HSE, March-Hugstetten, FRG). Isotonic longitudinal contractions were registered on a Watanabe multicorder. The temperature of the bath was changed within 3 s from 37°C to 27°C or from 27°C to 37°C in 2 min intervals according to Holzer & Lembeck (1979b). Drugs were administered simultaneously with the change of temperature from 37°C to 27°C. Administration of a drug was made as soon as the contraction induced by cooling had completely returned to its predrug height after the previous addition of an opioid substance.

Drugs

[Leu] enkephalin (Sigma), δ-receptor peptide, dynorphin-(1–13), dynorphin-(1–17) (Peninsula), dermorphin (gift of Prof. Erspamer), FK 33-824 [D-Ala^2-Mephe^4-Met(0)^5-ol]enkephalin (Sandoz) and morphine (Merck) were used.

Results

Effects of opioids on peristalsis

The peristaltic contractions propelled a constant amount of fluid during the periods of raised intraluminal pressure for 3 to 4 h. The intraarterial infusion of opioids reduced the efficiency of peristalsis dose-dependently as measured by the amount of fluid expelled (Figure 1). At lower concentrations, the frequency and/or pressure amplitude of the peristaltic waves were diminished. Higher concentrations led to incomplete segmental contractions without propagation of a peristaltic wave or to complete paralysis of the gut (Figure 2). Dynorphin-(1–17) caused a more pronounced inhibition of the peristaltic activity than

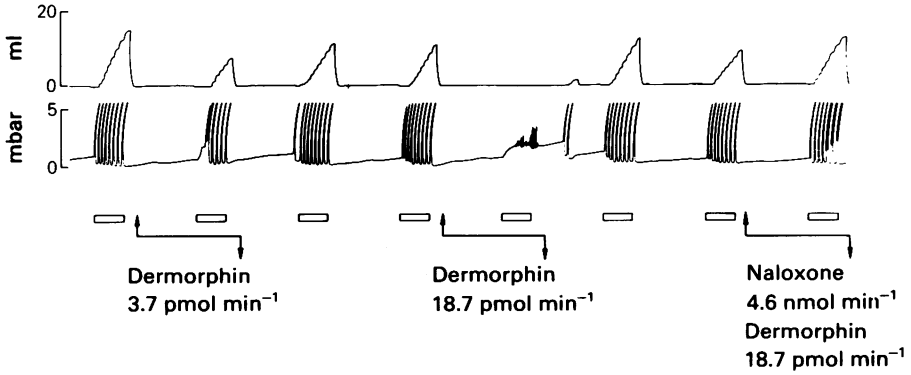

Figure 2 Tracing showing the effects of intraarterial infusion of dermorphin and dermorphin plus naloxone on the peristalsis of the guinea-pig ileum. Upper panel: volume of fluid propelled. Lower panel: aboral intraluminal pressure. Horizontal bars below the tracing indicate 150 s periods during which intraluminal pressure was raised from 0 to 2.5 mBar.

Table 1 Effect of naloxone on opioid inhibition of intestinal peristalsis

	% volume of fluid expelled		
		Naloxone	
	Without naloxone	*4.6 nmol min^{-1}*	*46 nmol min^{-1}*
Dermorphin: 18.7 pmol min^{-1}	0 ± 0	81 ± 6	–
FK 33-824: 102.4 pmol min^{-1}	13 ± 13	101 ± 8	–
Dynorphin-(1–13): 935.2 pmol min^{-1}	12 ± 12	22 ± 15	98 ± 7
Dynorphin-(1–17): 68.8 pmol min^{-1}	10 ± 4	102 ± 1	–
δ-Receptor peptide: 2183.4 pmol min^{-1}	9 ± 9	111 ± 7	–
Morphine: 7017.5 pmol min^{-1}	15 ± 13	104 ± 4	–

Propulsive peristaltic activity of the guinea-pig ileum is expressed as % volume of fluid propelled in the period of drug infusion compared with the period immediately before drug infusion (100%). Means ± s.e.mean; $n = 4$.

dynorphin-(1–13). Dermorphin was found to be the most potent inhibitor of peristalsis.

The inhibitory effects of opioids (at the highest dose used) were found to be susceptible to blockade by naloxone (simulataneous infusion of 4.6–46 nmol min^{-1} naloxone, Table 1). Naloxone itself (4.6–46 nmol min^{-1}) was devoid of any effect on peristalsis ($n = 4$).

Effect of opioids on cooling-induced contraction

The rapid change of the bath temperature from 37°C to 27°C induced a longitudinal contraction of the ileum. The initial phase of contraction was interrupted by an intermediate relaxation; the contraction reached its maximum about 20–25 s after the change of temperature as described by Holzer & Lembeck (1979b). As shown in Figure 3, opioids inhibited the cooling-induced contraction dose-dependently. Dermorphin, dynorphin-(1–13) and FK 33-824 were nearly equipotent. The inhibition of cooling-induced contractions caused by the highest dose of each opioid was antagonized by naloxone 5.1 μM, except that of dynorphin-(1–13), which could be antagonized only by 51 μM naloxone ($n = 4$).

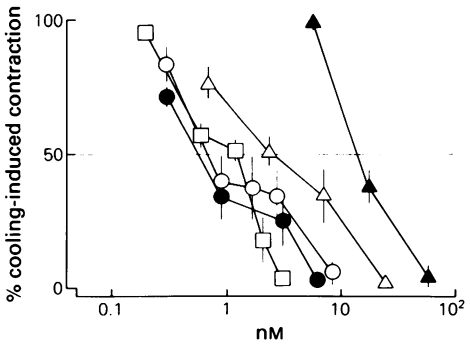

Figure 3 Effect of morphine and opioid peptides on cooling induced contraction of the guinea-pig ileum. All drugs were added simultaneously with rapid cooling. Symbols as in Figure 1. Means of $n = 4–6$; vertical lines show s.e.mean.

Discussion

The observation that the inhibitory effects of the opioids could be prevented by naloxone supports the involvement of specific opioid receptors in this inhibition. The longitudinal contractions induced by rapid cooling are caused by excitation of postganglionic fibres (Holzer & Lembeck, 1979b). The rank order of potency in inhibiting cooling-induced contractions was similar to the rank order in inhibiting contractions induced by electrical field stimulation (Erspamer *et al.*, 1981; Yoshimura *et al.*, 1982). However, the rank order of potency was not identical when cooling-induced contractions and peristalsis were compared. Generally there were greater differences in the ability of opioids to inhibit peristaltic activity. In the peristaltic reflex, there are at least two excitatory pathways, acetylcholine being the neuroeffector transmitter in one of these pathways and substance P being involved in the atropine-resistant peristalsis (Barthó *et al.*, 1982b). Opioids inhibit not only the release of acetylcholine (Szerb, 1982; Yau *et al.*, 1983) but also the release of substance P from enteric neurones (Holzer, 1984; Donnerer *et al.*, 1984).

First, it may be inferred from our results that the opioid agonists have different relative potencies on the (at least) two excitatory pathways of peristalsis. Second, the peristaltic reflex, which is a complex array of circular and longitudinal muscle contractions and relaxations, seems to be much more suitable for the differentiation of opioid activity in the gut than is the monitoring of longitudinal muscle contractions and may provide a more physiological model.

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