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PRESYNAPTIC OPIOID δ-RECEPTORS IN THE RABBIT MESENTERIC ARTERY

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SUMMARY

1. Excitatory junction potentials (e.j.p.s) evoked by nerve stimulation were recorded from muscle cells of the rabbit isolated mesenteric artery. At 0·03 Hz the e.j.p. amplitudes were stable. When a train of fifteen pulses was applied at 0·25 Hz or at higher frequencies (0·5, 1 and 2 Hz), e.j.p.s showed an initial facilitation followed by depression.

2. [Met^5]enkephalin 0·1 and 1 μmol/l, [D-Ala^2,D-Leu^5]enkephalin 0·1 and 1, but not 0·01 μmol/l, and [D-Pen^2, L-Pen^5]enkephalin 3 μmol/l all depressed the e.j.p.s evoked by trains of fifteen pulses at 1 Hz. When more than one concentration was used ([Met^5]enkephalin, [D-Ala^2,D-Leu^5]enkephalin), the inhibition was concentration dependent. It was always greater for the first few e.j.p.s than for the later ones in a train. [Met^5]enkephalin 1 μmol/l reduced the first e.j.p. at 1 Hz and the e.j.p.s evoked by 0·03 Hz to a similar extent.

3. The inhibitory effect of [Met^5]enkephalin 1 μmol/l on e.j.p.s persisted in the presence of yohimbine 0·3 μmol/l. Naloxone 1 μmol/l did not interfere with the effect of [Met^5]enkephalin 1 μmol/l. Naloxone 10 μmol/l depressed some e.j.p.s and prevented the inhibition by [Met^5]enkephalin 1 μmol/l. Neither ICI 154129 10 μmol/l nor ICI 174864 0·3 μmol/l had any effect of their own and both compounds antagonized the action of [Met^5]enkephalin 1 μmol/l.

4. Normorphine 10 μmol/l, fentanyl 1 μmol/l, ethylketocyclazocine 0·1 μmol/l, and dynorphin A(1–13) 1 μmol/l were all ineffective. Ethylketocyclazocine 1 μmol/l did not change the e.j.p.s either, but antagonized [Met^5]enkephalin 1 μmol/l.

5. [Met^5]enkephalin 1 μmol/l failed to influence both the resting membrane potential of the muscle cells and the depolarizing effect of noradrenaline 3 and 30 μmol/l.

6. We suggest that the axon terminals of post-ganglionic sympathetic neurones in the rabbit mesenteric artery possess opioid δ-, but not μ- or κ-receptors. The activation of presynaptic δ-receptors inhibits the release of the neuroeffector transmitter. There is no evidence for any effect of co-released endogenous opioid peptides under our experimental conditions.

INTRODUCTION

Opioids decrease blood pressure mainly by acting at cardiovascular centres in the brain (Holaday, 1983; McQueen, 1983). However, peripheral mechanisms, e.g. the activation of inhibitory receptors situated at post-ganglionic sympathetic nerve terminals in blood vessels, may be also involved in the hypotensive effect of opioids (Illes & Pfeiffer, 1985). The presence of such presynaptic receptors (Starke, 1977; Henderson, Hughes & Kosterlitz, 1979) has been demonstrated in the rabbit ear artery (Knoll, 1976; Ronai, Harsing, Berzetei, Bajusz & Vizi, 1982; Illes, Pfeiffer, von Kügelgen & Starke, 1985*a*) and ileocolic artery (von Kügelgen, Illes, Wolf & Starke, 1985). Except in some cerebral arteries (Hanko & Hardebo, 1978; Altura, Altura & Quirion, 1984; Harder & Madden, 1984) opioids do not cause vasodilation by a direct effect on the smooth muscle, although they may attenuate noradrenaline-induced vasoconstriction (Ruth, Doerr & Eiden, 1984).

In smooth muscle organs the stimulation of post-ganglionic sympathetic fibres evokes excitatory junction potentials (e.j.p.s). The e.j.p. amplitudes are a measure of transmitter release per pulse (Burnstock & Bell, 1974). It has been shown that in the mouse vas deferens opioids depress the release of noradrenaline (Henderson, Hughes & Kosterlitz, 1972; Hughes, Kosterlitz & Leslie, 1975), and in consequence e.j.p.s (Henderson & North, 1976; Illes, Zieglgänsberger & Herz, 1980). The nerve terminals of the mouse vas deferens possess all three types of opioid receptor (Wüster, Schulz & Herz, 1981).

The effect of opioids on vascular sympathetic neuroeffector transmission has not yet been studied electrophysiologically. In the ear artery (Illes *et al.* 1985*a*) and ileocolic artery of rabbits (von Kügelgen *et al.* 1985) the measurement of stimulation-evoked vasoconstriction and noradrenaline overflow suggests the presence of presynaptic δ- and κ-, but not μ-receptors. Since small-diameter arteries may be more important for blood pressure regulation than these relatively large vessels, we have chosen peripheral branches of the rabbit mesenteric artery for the present study. We recorded e.j.p.s from individual smooth muscle cells and tested the effect of receptor type selective opioids. A brief report of some results has been published (Illes, Ramme & Starke, 1985*b*).

METHODS

Preparation and recording

Rabbits of either sex (1·5–2·5 kg) were decapitated. Jejunal branches of the superior mesenteric artery were excised together with the parallel lymph vessels and veins. After the surrounding connective tissue had been stripped off, arteries with an external diameter of 0·2–0·4 mm and about 5 mm long were pinned to the bottom of a 1·5 ml organ bath. The preparation was superfused with Krebs solution (in mmol/l: NaCl, 118; KCl, 4·8; $CaCl_2$, 2·5; $MgSO_4$, 1·2; KH_2PO_4, 0·9; $NaHCO_3$, 25 and glucose, 11) saturated with 95% O_2 and 5% CO_2 and maintained at 37 °C. The flow rate was 1 ml/min.

Intracellular potentials were recorded from smooth muscle cells with glass micro-electrodes filled with KCl 2·5 mol/l and having a resistance of 50–90 MΩ. A WPI M-707 pre-amplifier coupled to a Tektronix 5113 oscilloscope and a Watanabe WTR 311 pen recorder was used for registration. The membrane potential of single cells was between 60 and 70 mV.

Nerve stimulation-evoked e.j.p.s

The perivascular nerves were stimulated with bipolar platinum electrodes placed perpendicularly to the length of the organ and 0·5 mm apart. The site of recording was between the two stimulating electrodes. Pulses of 1 ms duration and 1–20 V intensity were supplied by the stimulus isolation unit of a Hugo Sachs Stimulator II. The voltage was adjusted so that an e.j.p. of approximately 10 mV amplitude was evoked by a single pulse. The e.j.p.s were abolished by tetrodotoxin 0·5 μmol/l. Stimulation was either with single pulses every 30 s (0·03 Hz) or with trains of fifteen pulses at 1 Hz every 3 min. In some experiments the frequency of stimulation was increased stepwise from 0·25 to 0·5, 1 and 2 Hz; fifteen pulses were applied at each frequency. The trains were spaced 3 min apart. Drug effects were evaluated as percentage inhibition. Agonist-induced changes refer to the second pulse train in the presence of agonist (6 min after addition) as compared to the last pulse train before agonist. Antagonist-induced changes refer to the third (yohimbine second) pulse train in the presence of antagonist (9 min or yohimbine 6 min after addition) as compared to the last pre-antagonist pulse train. At 0·03 Hz the amplitudes of the eleventh to fourteenth e.j.p. after agonist addition were averaged (6–7 min contact time) and were compared to the average of the five pre-agonist e.j.p. amplitudes. In order to determine whether e.j.p.s changed with time, measurements were carried out also in the absence of drugs.

Noradrenaline-induced depolarization

When the effect of [Met^5]enkephalin on resting membrane potential and noradrenaline-induced depolarization was determined, ascorbic acid 0·3 mmol/l and Na_2EDTA 0·03 mmol/l were present in the Krebs solution. Measurements were carried out while the tissue was superfused in the following order: drug-free medium; medium containing noradrenaline 3 or 30 μmol/l (for 12–15 min); drug-free medium (for 47–50 min, during which the membrane potential recovered to its original level); medium containing [Met^5]enkephalin 1 μmol/l (for 12–15 min); and medium containing both [Met^5]enkephalin and noradrenaline, at the same concentration as before. In each solution, the membrane potential of five cells was measured, a procedure that took 7–10 min. The first cell was impaled after a drug contact of 6 min (or after 40 min of wash-out). The average membrane potential of five cells was calculated in solutions containing noradrenaline and/or [Met^5]enkephalin; measurements during the two drug-free periods (ten cells) were also averaged.

Materials

The following drugs were used; [D-Ala^2,D-Leu^5]enkephalin formate, [D-Pen^2,L-Pen^5]enkephalin (Bachem, Bubendorf, Switzerland); dynorphin A(1–13) (Sigma, München, F.R.G.); ethylketocyclazocine methansulphonate (Dr A. E. Soria, Sterling-Winthrop Research Institute, Rensselaer, U.S.A.); fentanyl citrate (Janssen, Beerse, Belgium); [Met^5]enkephalin acetate (Sigma, München, F.R.G.); ICI 154129 (*N*,*N*-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH), ICI 174864 (*N*,*N*-diallyl-Tyr-α-aminoisobutyric acid-α-aminoisobutyric acid-Phe-Leu-OH; Dr J. S. Shaw, Imperial Chemical Industries, Macclesfield, U.K.); naloxone hydrochloride (Endo, Garden City, U.S.A.); normorphine hydrochloride (Bios Products, Brussels, Belgium); tetrodotoxin (Sigma, München, F.R.G.); yohimbine hydrochloride (Roth, Karlsruhe, F.R.G.).

Stock solutions (1–10 mmol/l) of all drugs were prepared with distilled water; those of the opioid peptides were stored at −20 °C. Further dilutions were made in Krebs solution.

Statistics

Means ± S.E. of mean are given throughout the paper. Student's *t* test was used for comparison of the means, as well as for comparison of experimentally determined means with a hypothetical mean value of zero. A probability level of 0·05 and less was considered to be statistically significant.

RESULTS

Different stimulation frequencies

E.j.p.s evoked by single stimuli (0·03 Hz) did not vary (Fig. 1*A*). At higher frequencies (0·25, 0·5, 1 and 2 Hz) an initial facilitation was followed by depression (Fig. 1*A* and *B*). At 2 Hz partial summation occurred, since the depolarization still

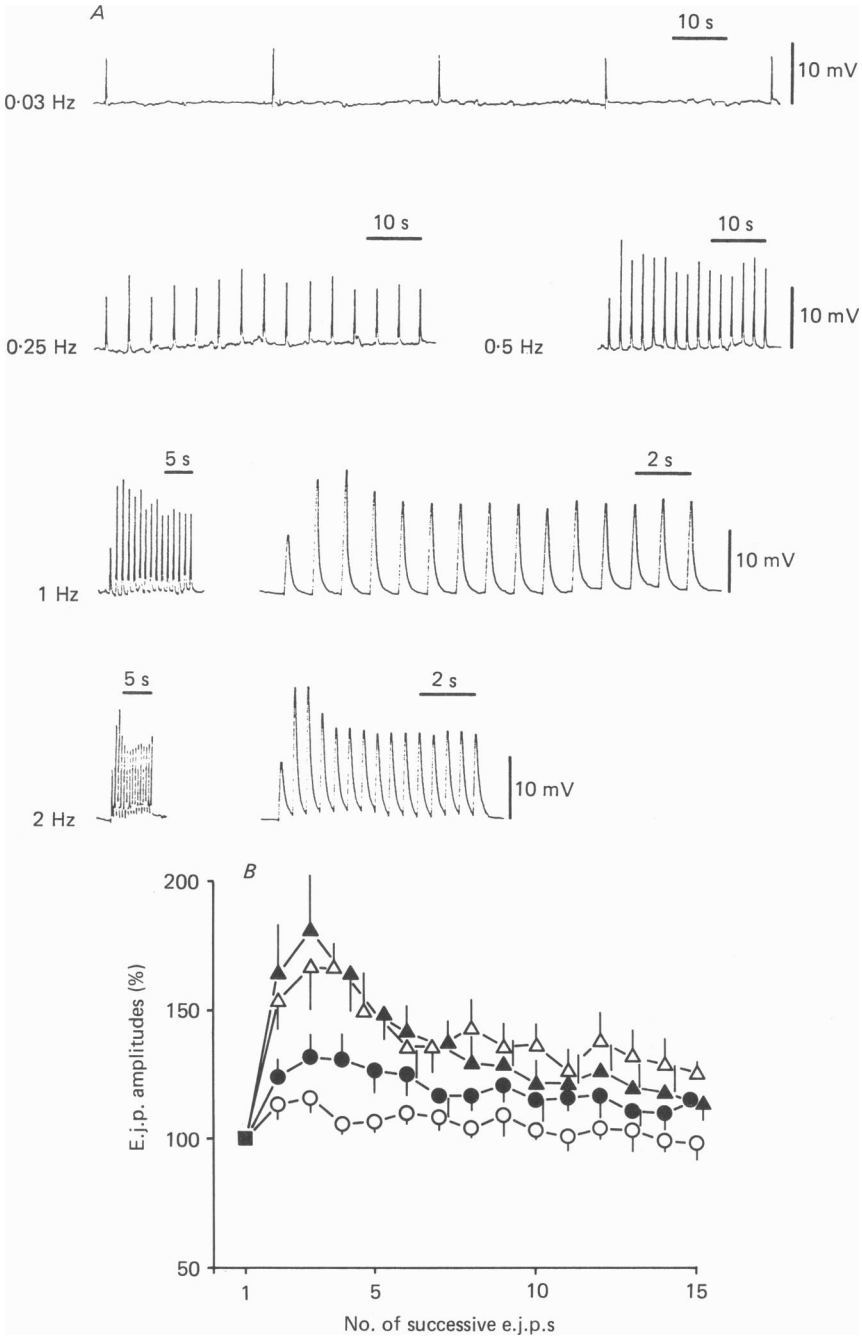

Fig. 1. E.j.p.s evoked with different frequencies of stimulation in the rabbit mesenteric artery. *A*, representative tracings. *B*, facilitation and depression of fifteen e.j.p.s at 0·25 (○), 0·5 (●), 1 (△) and 2 Hz (▲). Mean ± S.E. of five experiments. The amplitude of the first e.j.p. at each frequency was taken as 100 %; it was 11·1 ± 0·6 mV (○), 9·9 ± 0·6 mV (●), 9·3 ± 1·2 mV (△) and 9·4 ± 1·0 mV (▲).

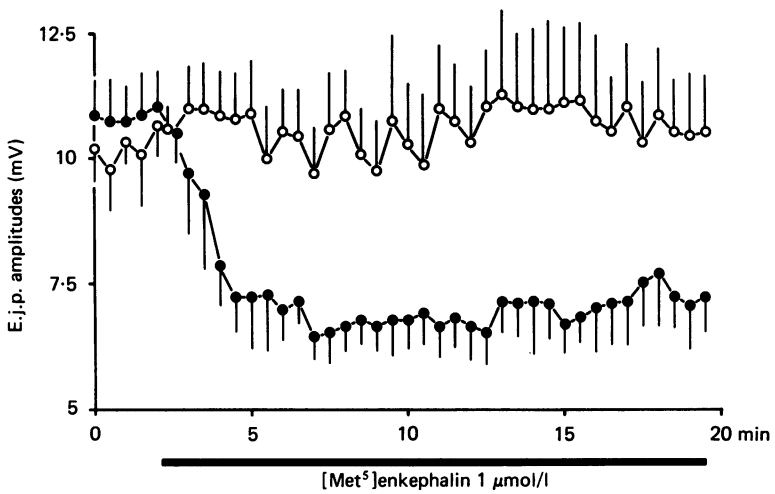

Fig. 2. Constancy of control (○) e.j.p. amplitudes and their depression by [Met⁵]enkephalin 1 μmol/l (●). The e.j.p.s were evoked by a frequency of 0·03 Hz. [Met⁵]enkephalin was added as indicated by the continuous bar. Mean ± S.E. of four experiments.

persisted at the onset of the next e.j.p. In the following experiments, we used two frequencies at which either no facilitation (0·03 Hz) or maximum facilitation without summation (1 Hz) was observed.

Effect of [Met⁵]enkephalin on e.j.p.s

Stimulation for 20 min at 0·03 Hz continued to evoke stable e.j.p.s (Fig. 2). [Met⁵]enkephalin 1 μmol/l depressed the e.j.p. amplitudes by 37·8 ± 6·5% after 6–7 min ($n = 4$; $P < 0·05$); the inhibition caused by this concentration was near maximal in the third minute of drug application. The inhibitory effect did not fade with time.

In the following experiments trains of fifteen pulses at 1 Hz were applied every 3 min. In the absence of drugs, the e.j.p. amplitudes did not change over a period of 6 min (Table 1). [Met⁵]enkephalin 0·1 μmol/l depressed only the first two e.j.p.s in the train but the subsequent ones were not affected (Table 1). A higher concentration of [Met⁵]enkephalin (1 μmol/l) depressed all e.j.p.s; the percentage inhibition of the first two e.j.p.s was more marked than that of the subsequent ones (Fig. 3*B*, Table 1). The opioid effect was reversed by wash-out (Fig. 3*A*).

Interaction between yohimbine and [Met⁵]enkephalin

Yohimbine 0·3 μmol/l had no consistent effect on the first three e.j.p.s in a train, but enhanced the amplitude of the subsequent ones (Fig. 4*A*, Table 1). A higher concentration of yohimbine (1 μmol/l) caused still larger facilitation and a marked depression of the first e.j.p. (not shown). [Met⁵]enkephalin 1 μmol/l inhibited the e.j.p.s in the presence of yohimbine 0·3 μmol/l; the percentage inhibition of the first and fifteenth e.j.p. was similar to that observed in the absence of yohimbine (Table 1). However, whereas the effect of [Met⁵]enkephalin decreased abruptly after

TABLE 1. Effect of opioids and yohimbine on the first, second and fifteenth e.j.p. elicited by trains of fifteen pulses at 1 Hz

Treatment (μmol/l)		n	Inhibition (%) First e.j.p. amplitude	Second e.j.p. amplitude	Fifteenth e.j.p. amplitude
None		5	6·9 ± 2·9	−5·8 ± 5·5	−3·0 ± 5·2
[Met⁵]enkephalin	0·1	5	31·8 ± 6·7**	20·8 ± 5·8*	−0·4 ± 2·6††
	1·0	5	36·4 ± 4·0***	38·8 ± 6·3**	18·2 ± 4·7*†
Yohimbine	0·3	7	2·7 ± 1·0*	8·9 ± 4·4	−22·4 ± 6·2*†
+ [Met⁵]enkephalin	1·0	7	32·5 ± 5·5**	27·4 ± 7·1**	18·1 ± 3·0***†
Naloxone	1·0	5	6·5 ± 9·2	1·6 ± 5·6	7·8 ± 4·5
+ [Met⁵]enkephalin	1·0	5	30·7 ± 6·3**	27·3 ± 5·0**	15·3 ± 2·5**†
Naloxone	10·0	5	14·3 ± 5·3	19·7 ± 5·2*	8·2 ± 5·4
+ [Met⁵]enkephalin	1·0	5	12·8 ± 3·4*‡	10·4 ± 2·8*‡	10·7 ± 4·1
ICI 154129	10·0	5	2·5 ± 2·7	−5·1 ± 5·5	−4·9 ± 2·9
+ [Met⁵]enkephalin	1·0	5	4·2 ± 3·0‡‡	7·0 ± 5·1‡	8·3 ± 5·1
ICI 174864	0·3	5	−4·2 ± 6·2	−13·1 ± 10·6	−10·8 ± 6·9
+ [Met⁵]enkephalin	1·0	5	13·7 ± 2·5**‡‡	11·8 ± 6·1‡	4·6 ± 5·0
[D-Ala²,D-Leu⁵]enkephalin	0·01	5	−2·9 ± 10·8	6·8 ± 9·6	3·9 ± 11·1
	0·1	6	34·0 ± 6·5**	32·1 ± 9·4*	25·2 ± 7·4*
	1·0	5	38·6 ± 4·3***	42·1 ± 7·7**	23·7 ± 7·5*†
[D-Pen²,L-Pen⁵]enkephalin	3·0	5	29·0 ± 3·3***	26·2 ± 2·7***	9·9 ± 3·6†
Normorphine	10·0	5	9·4 ± 4·7	13·0 ± 6·8	5·5 ± 4·2
Fentanyl	1·0	5	4·5 ± 8·4	1·8 ± 7·0	−13·0 ± 6·9
Ethylketocyclazocine	0·1	5	13·4 ± 15·6	14·5 ± 11·7	8·7 ± 13·5
	1·0	5	−7·3 ± 11·3	−1·3 ± 6·4	−12·9 ± 10·9
Ethylketocyclazocine	1·0	5	15·5 ± 6·5	9·3 ± 4·0	1·6 ± 3·3
+ [Met⁵]enkephalin	1·0	5	8·0 ± 2·7*‡‡	9·7 ± 4·1‡	2·0 ± 3·5
Dynorphin A(1–13)	1·0	5	16·0 ± 6·9	7·5 ± 9·3	−0·7 ± 6·1

In interaction experiments, yohimbine, naloxone, ICI 154129 and ethylketocyclazocine were given first and [Met⁵]enkephalin was added 6 min (yohimbine, ethylketocyclazocine) or 9 min (naloxone, ICI 154129, ICI 174864) later; the percentage inhibition was evaluated by comparison with the last pulse train before [Met⁵]enkephalin (but in the presence of antagonist).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; significant differences from zero.

† $P < 0.05$; †† $P < 0.01$; significant differences from 1st e.j.p.

‡ $P < 0.05$; ‡‡ $P < 0.01$; significant differences from [Met⁵]enkephalin 1 μmol/l alone.

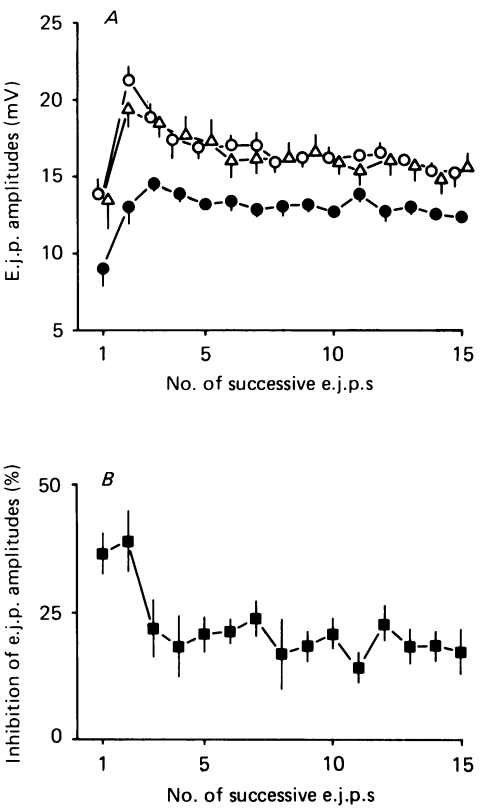

Fig. 3. Effect of [Met^5]enkephalin on the amplitude of e.j.p.s evoked by trains of fifteen pulses at 1 Hz. *A*, ○, before drug addition; ●, [Met^5]enkephalin 1 μmol/l; △, after wash-out for 20 min. Mean ± S.E. of five experiments. *B*, percentage inhibition of e.j.p. amplitudes by [Met^5]enkephalin 1 μmol/l, calculated from the data of ● and ○ in *A*.

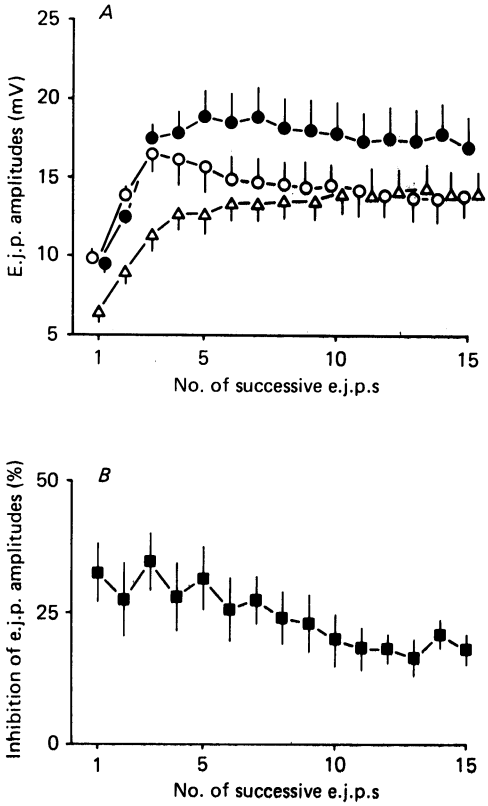

Fig. 4. Effect of [Met^5]enkephalin in the presence of yohimbine on the amplitude of e.j.p.s evoked by trains of fifteen pulses at 1 Hz. *A*, ○, before drug addition; ●, yohimbine 0·3 μmol/l; △, yohimbine 0·3 μmol/l + [Met^5]enkephalin 1 μmol/l. Mean ± S.E. of seven experiments. *B*, percentage inhibition of e.j.p. amplitudes by [Met^5]enkephalin, calculated from the data of △ and ● in *A*.

the second e.j.p. in the absence of yohimbine (Fig. 3*B*), it declined more smoothly when yohimbine was present (Fig. 4*B*).

Interaction between opioid antagonists and [Met^5]enkephalin

Naloxone 1 μmol/l had no effect on the e.j.p.s by itself and did not influence the inhibition by [Met^5]enkephalin 1 μmol/l (Fig. 5*A*, Table 1). A higher concentration of naloxone (10 μmol/l) tended to depress all e.j.p. amplitudes (Fig. 5*B*), although only some e.j.p.s were inhibited significantly (Table 1). Naloxone 10 μmol/l almost abolished the inhibitory action of [Met^5]enkephalin 1 μmol/l. ICI 154129 10 μmol/l and ICI 174864 0·3 μmol/l both antagonized the [Met^5]enkephalin inhibition, without having any effect of their own (Fig. 6, Table 1).

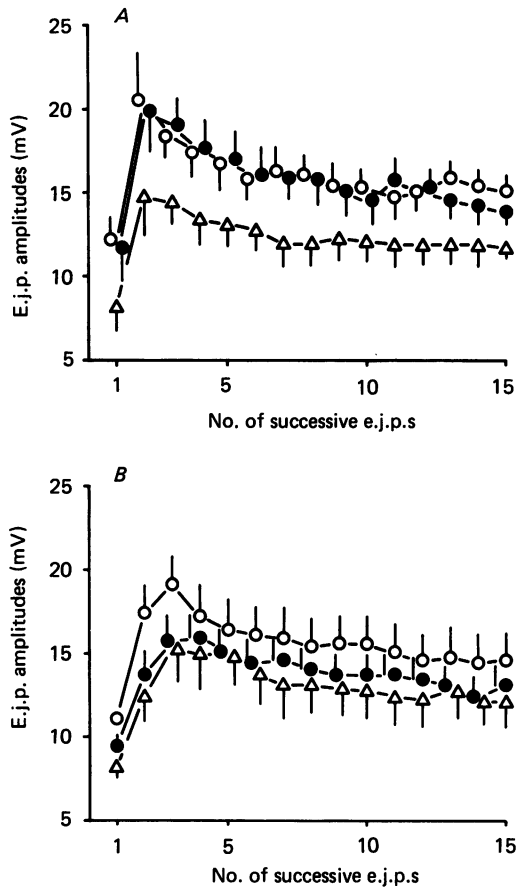

Fig. 5. The effect of [Met^5]enkephalin in the presence of naloxone on the amplitude of e.j.p.s evoked by trains of fifteen pulses at 1 Hz. *A*, ○, before drug addition; ●, naloxone 1 μmol/l; △, naloxone 1 μmol/l + [Met^5]enkephalin 1 μmol/l. Mean ± S.E. of five experiments. *B*, ○, control; ●, naloxone 10 μmol/l; △, naloxone 10 μmol/l + [Met^5]enkephalin 1 μmol/l. Mean ± S.E. of mean of five experiments.

Effect of other opioid agonists

[D-Ala^2,D-Leu^5]enkephalin 0·1 and 1 μmol/l, but not 0·01 μmol/l depressed the e.j.p.s (Table 1). [D-Pen^2,L-Pen^5]enkephalin 3 μmol/l caused an inhibition comparable to that produced by [Met^5]enkephalin 0·1 μmol/l. Normorphine 10 μmol/l, fentanyl 1 μmol/l, ethylketocyclazocine 0·1 μmol/l and dynorphin A(1–13) 1 μmol/l were ineffective. Ethylketocyclazocine 1 μmol/l did not change the e.j.p.s either, but antagonized the action of [Met^5]enkephalin 1 μmol/l.

Effect of [Met^5]enkephalin on noradrenaline-induced depolarization

[Met^5]enkephalin 1 μmol/l failed to influence the resting membrane potential of muscle cells (63·6 ± 1·0 mV in the absence and 62·3 ± 1·2 mV in the presence of the opioid; ten experiments; $P > 0·05$). Similarly, [Met^5]enkephalin 1 μmol/l did not

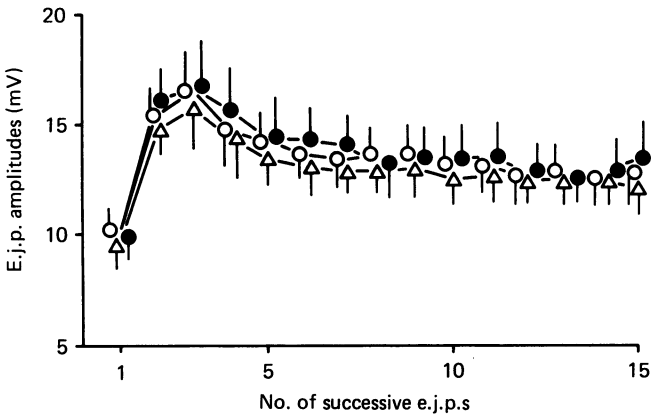

Fig. 6. The effect of [Met^5]enkephalin in the presence of ICI 154129 on the amplitude of e.j.p.s evoked by trains of fifteen pulses at 1 Hz. ○, before drug addition; ●, ICI 154129 10 μmol/l; △, ICI 154129 10 μmol/l + [Met^5]enkephalin 1 μmol/l. Mean ± S.E. of five experiments.

change the depolarizing effect of noradrenaline 3 μmol/l (5·0 ± 1·5 mV before and 6·3 ± 1·4 mV after opioid application; five experiments; $P > 0.05$) or of noradrenaline 30 μmol/l (14·6 ± 1·6 mV before and 14·5 ± 1·6 mV after opioid application; five experiments; $P > 0.05$).

DISCUSSION

Presence of δ-receptors

The main finding of the present study is that certain opioid peptides inhibit neuroeffector transmission in jejunal branches of the rabbit mesenteric artery. [Met^5]enkephalin and [D-Ala^2,D-Leu^5]enkephalin are somewhat selective for opioid δ-receptors (Lord, Waterfield, Hughes & Kosterlitz, 1977; Paterson, Robson & Kosterlitz, 1983); [D-Pen^2,L-Pen^5]enkephalin is a highly selective δ-agonist (Mosberg, Hurst, Hruby, Gee, Yamamura, Galligan & Burks, 1983). All three peptides reduced the amplitude of nerve stimulation-evoked e.j.p.s. Naloxone, a preferential μ-antagonist (Lord *et al.* 1977; Paterson *et al.* 1983) failed to influence the effect of [Met^5]enkephalin at the lower concentration (1 μmol/l) applied. At the higher concentration of 10 μmol/l it reduced the e.j.p.s and also antagonized the inhibition by [Met^5]enkephalin. We did not clarify the mechanism of the depressive effect of naloxone on the e.j.p.s; however, it does not seem to be mediated by opioid receptors. Finally, inhibition by [Met^5]enkephalin was diminished by the δ-selective antagonists ICI 154129 (Shaw, Miller, Turnbull, Gormley & Morley, 1982) and ICI 174864 (Cotton, Giles, Miller, Shaw & Timms, 1984) in concentrations (10 and 0·3 μmol/l, respectively), which did not influence neuroeffector transmission.

Absence of μ- and κ-receptors

The selective μ-agonists normorphine and fentanyl (Gillan, Kosterlitz & Paterson, 1980; Paterson *et al.* 1983) did not depress the e.j.p. amplitudes in the mesenteric

artery. The same was true for the preferential κ-agonists ethylketocyclazocine (Magnan, Paterson, Tavani & Kosterlitz, 1982) and dynorphin A(1–13) (Corbett, Paterson, McKnight, Magnan & Kosterlitz, 1982), although both substances, at least in some experimental groups, tended to reduce the early e.j.p.s in the train (Table 1). However, ethylketocyclazocine abolished the depression of e.j.p.s by [Met^5]enkephalin, suggesting that this benzomorphan has some antagonist action at the δ-receptor. A similar effect of ethylketocyclazocine was shown in the hamster vas deferens, another organ containing only presynaptic δ-receptors (McKnight, Corbett, Marcoli & Kosterlitz, 1984).

The lack of activity of μ-agonists in the mesenteric artery agrees but the lack of activity of κ-agonists contrasts with the results obtained in the ear artery (Illes *et al.* 1985*a*) and the ileocolic artery of rabbits (von Kügelgen *et al.* 1985). These larger arteries contained both δ- and κ-, but no μ-receptors. In pithed rabbits, with electrical stimulation of sympathetic outflow, ethylketocyclazocine and bremazocine decreased the release of noradrenaline and lowered blood pressure, probably as a consequence of the activation of presynaptic κ-receptors (Ensinger, Hedler, Schurr & Starke, 1984; Ensinger, Hedler, Szabo & Starke, 1985). Possibly, κ-receptors are situated in the heart (Starke, Schöffel & Illes, 1985) and in large arteries (Illes *et al.* 1985*a*; von Kügelgen *et al.* 1985), but not in the small resistance vessels of the rabbit, as shown by the present finding.

Site of action

In the rabbit mesenteric artery [Met^5]enkephalin seems to inhibit the release of the neuroeffector transmitter rather than to interfere with a post-synaptic effect. Four observations are in favour of this interpretation. First, [Met^5]enkephalin does not change the resting membrane potential of the muscle cells. Secondly, it fails to influence the noradrenaline-induced depolarization. Thirdly, it causes a larger inhibition of the first e.j.p.s than of the subsequent e.j.p.s of a train; if the effect were post-synaptic all e.j.p.s should be inhibited to the same degree. Finally, in jejunal arteries preincubated with [^{3}H]noradrenaline, [Met^5]enkephalin depresses the electrically evoked overflow of tritium which is a direct index of noradrenaline release (Ramme, Illes, Späth & Starke, 1986).

It seems to be a general characteristic of presynaptic inhibition that it declines with the length of the train. This was demonstrated, e.g. for opioids (Illes *et al.* 1980) and α_2-agonists (Illes & Starke, 1983) in the mouse vas deferens, by an electrophysiological method similar to that used in the present experiments. We have now shown that [Met^5]enkephalin and also other δ-agonists inhibit the first two e.j.p.s in the train to a larger extent than the subsequent ones. However, the inhibitory effect of [Met^5]enkephalin decreases gradually when the α_2-adrenergic feed-back mechanism, which is probably responsible for the depression of the later e.j.p.s (Mishima, Miyahara & Suzuki, 1984), is excluded by the α_2-antagonist yohimbine.

Possible physiological function

Opioid peptides may be co-stored with noradrenaline in post-ganglionic sympathetic nerves (Wilson, Klein, Chang, Gasparis, Viveros & Yang, 1980; Lang, Hermann, Dietz, Gaida, Ganten, Kraft & Unger, 1983) and are present in perivascular nerves

of precapillary vessels (Lundberg, Hökfelt, Änggard, Uvnäs-Wallensten, Brimijoin, Brodin & Fahrenkrug, 1980). Hence, enkephalins released on nerve stimulation might inhibit neuroeffector transmission in the rabbit mesenteric artery. However, ICI 154129 and ICI 174864 in concentrations (10 and 0·3 μmol/l, respectively) sufficient to prevent the effect of [Met^5]enkephalin 1 μmol/l, did not change the e.j.p. amplitudes in the train. Moreover, naloxone 10 μmol/l reduced rather than enhanced the e.j.p.s. Thus, in contrast to the physiological α_2-adrenergic control of transmitter release (Mishima *et al.* 1984), no comparable endogenous opioid mechanism seems to operate under our experimental conditions.

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REFERENCES

ALTURA, B. T., ALTURA, B. M. & QUIRION, R. (1984). Identification of benzomorphan-κ opiate receptors in cerebral arteries which subserve relaxation. *British Journal of Pharmacology* **82**, 459–466.

BURNSTOCK, G. & BELL, C. (1974). Peripheral autonomic transmission. In *The Peripheral Nervous System*, ed. HUBBARD, J. I., pp. 277–327. New York: Plenum Press.

CORBETT, A. D., PATERSON, S. J., MCKNIGHT, A. T., MAGNAN, J. & KOSTERLITZ, H. W. (1982). Dynorphin$_{1-8}$ and dynorphin$_{1-9}$ are ligands for the κ-subtype of opiate receptor. *Nature* **299**, 79–81.

COTTON, R., GILES, M. G., MILLER, L., SHAW, J. S. & TIMMS, D. (1984). ICI 174864: a highly selective antagonist for the opioid δ-receptor. *European Journal of Pharmacology* **97**, 331–332.

ENSINGER, H., HEDLER, L., SCHURR, C. & STARKE, K. (1984). Ethylketocyclazocine decreases noradrenaline release and blood pressure in the rabbit at a peripheral opioid receptor. *Naunyn-Schmiedebergs Archives of Pharmacology* **328**, 20–23.

ENSINGER, H., HEDLER, L., SZABO, B. & STARKE, K. (1986). Bremazocine causes sympathoinhibition and hypotension in rabbits by activating peripheral κ-receptors. *Journal of Cardiovascular Pharmacology* (in the Press).

GILLAN, M. G. C., KOSTERLITZ, H. W. & PATERSON, S. J. (1980). Comparison of the binding characteristics of tritiated opiates and opioid peptides. *British Journal of Pharmacology* **70**, 481–490.

HANKO, J. H. & HARDEBO, J. E. (1978). Enkephalin-induced dilatation of pial arteries in vitro probably mediated by opiate receptors. *European Journal of Pharmacology* **51**, 295–297.

HARDER, D. R. & MADDEN, J. A. (1984). Cellular mechanisms of opiate receptor stimulation in cat middle cerebral artery. *European Journal of Pharmacology* **102**, 411–416.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H. W. (1972). A new example of a morphine-sensitive neuroeffector junction: adrenergic transmission in the mouse vas deferens. *British Journal of Pharmacology* **46**, 764–766.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H. W. (1979). Modification of catecholamine release by narcotic analgesics and opioid peptides. In *The Release of Catecholamines from Adrenergic Neurones*, ed. PATON, D. M., pp. 217–228. Oxford: Pergamon Press.

HENDERSON, G. & NORTH, R. A. (1976). Depression by morphine of excitatory junction potentials in the vas deferens of the mouse. *British Journal of Pharmacology* **57**, 341–346.

HOLADAY, J. W. (1983). Cardiovascular effects of endogenous opiate systems. *Annual Review of Pharmacology and Toxicology* **23**, 541–594.

HUGHES, J., KOSTERLITZ, H. W. & LESLIE, F. M. (1975). Effect of morphine on adrenergic transmission in the mouse vas deferens. Assessment of agonist and antagonist potencies of narcotic analgesics. *British Journal of Pharmacology* **53**, 371–381.

ILLES, P. & PFEIFFER, N. (1985). Regulation of blood pressure by peripheral opioid mechanisms. In *Vascular Neuroeffector Mechanisms*, ed. BEVAN, J. A., GODFRAIND, T., MAXWELL, R. A., STOCLET, J. C. & WORCEL, M., pp. 175–180. Amsterdam: Elsevier.

ILLES, P., PFEIFFER, N., VON KÜGELGEN, I. & STARKE, K. (1985*a*). Presynaptic opioid receptor subtypes in the rabbit ear artery. *Journal of Pharmacology and Experimental Therapeutics* **232**, 526–533.

ILLES, P., RAMME, D. & STARKE, K. (1985*b*). Inhibition of neuroeffector transmission in the rabbit mesenteric artery by [Met^5]enkephalin. *European Journal of Pharmacology* **107**, 397–398.

ILLES, P. & STARKE, K. (1983). An electrophysiological study of presynaptic α-adrenoceptors in the vas deferens of the mouse. *British Journal of Pharmacology* **78**, 365–373.

ILLES, P., ZIEGLGÄNSBERGER, W. & HERZ, A. (1980). Calcium reverses the inhibitory action of morphine on neuroeffector transmission in the mouse vas deferens. *Brain Research* **191**, 511–522.

KNOLL, J. (1976). neuronal peptide (enkephalin) receptors in the ear artery of the rabbit. *European Journal of Pharmacology* **39**, 403–407.

LANG, R. E., HERMANN, K., DIETZ, R., GAIDA, W., GANTEN, D., KRAFT, K. & UNGER, T. (1983). Evidence for the presence of enkephalins in the heart. *Life Sciences* **32**, 399–406.

LORD, J. A. H., WATERFIELD, A. A., HUGHES, J. & KOSTERLITZ, H. W. (1977). Endogenous opioid peptides: multiple agonists and receptors. *Nature* **267**, 495–499.

LUNDBERG, J. M., HÖKFELT, T., ÄNGGARD, A., UVNÄS-WALLENSTEN, K., BRIMIJOIN, S., BRODIN, E. & FAHRENKRUG, J. (1980). Peripheral peptide neurons: distribution, axonal transport, and some aspects on possible function. *Advances in Biochemical Psychopharmacology* **22**, 25–36.

MCKNIGHT, A. T., CORBETT, A. D., MARCOLI, M. & KOSTERLITZ, H. W. (1984). Hamster vas deferens contains δ-opioid receptors. *Neuropeptides* **5**, 97–100.

MCQUEEN, D. S. (1983). Opioid peptide interactions with respiratory and circulatory systems. *British Medical Bulletin* **39**, 77–82.

MAGNAN, J., PATERSON, S. J., TAVANI, A. & KOSTERLITZ, H. W. (1982). The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. *Naunyn-Schmiedebergs Archives of Pharmacology* **319**, 197–205.

MISHIMA, S., MIYAHARA, H. & SUZUKI, H. (1984). Transmitter release modulated by α-adrenoceptor antagonists in the rabbit mesenteric artery: a comparison between noradrenaline outflow and electrical activity. *British Journal of Pharmacology* **83**, 537–547.

MOSBERG, H. I., HURST, R., HRUBY, V. J., GEE, K., YAMAMURA, H. I., GALLIGAN, J. J. & BURKS, T. F. (1983). Bis-penicillamine enkephalins possess highly improved specificity towards opioid receptors. *Proceedings of the National Academy of Sciences of the U.S.A.* **80**, 5871–5874.

PATERSON, S. J., ROBSON, L. E. & KOSTERLITZ, H. W. (1983). Classification of opioid receptors. *British Medical Bulletin* **39**, 31–36.

RAMME, D., ILLES, P., SPÄTH, L. & STARKE, K. (1986). Blockade of α_2-adrenoceptors permits the operation of otherwise silent opioid κ-receptors at the sympathetic axons of rabbit jejunal arteries. *Naunyn-Schmiedebergs Archives of Pharmacology* (in the Press).

RONAI, A. Z., HARSING, L. G., BERZETEI, I. P., BAJUSZ, S. & VIZI, E. S. (1982). [Met^5]enkephalin-Arg-Phe acts on vascular opiate receptors. *European Journal of Pharmacology* **79**, 337–338.

RUTH, J. A., DOERR, A. L. & EIDEN, L. E. (1984). [Leu^5]enkephalin inhibits norepinephrine-induced contraction of rat aorta. *European Journal of Pharmacology* **105**, 189–191.

SHAW, J. S., MILLER, L., TURNBULL, M. J., GORMLEY, J. J. & MORLEY, J. S. (1982). Selective antagonists at the opiate delta-receptor. *Life Sciences* **31**, 1259–1262.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Reviews of Physiology, Biochemistry and Pharmacology* **77**, 1–124.

STARKE, K., SCHÖFFEL, E. & ILLES, P. (1985). The sympathetic axons innervating the sinus node of the rabbit possess presynaptic opioid κ- but not μ- or δ-receptors. *Naunyn-Schmiedebergs Archives of Pharmacology* **329**, 206–209.

WILSON, S. P., KLEIN, R. L., CHANG, K.-J., GASPARIS, M. S., VIVEROS, O. H. & YANG, W.-H. (1980). Are opioid peptides co-transmitters in noradrenergic vesicles of sympathetic nerves? *Nature* **288**, 707–709.

WÜSTER, M., SCHULZ, R. & HERZ, A. (1981). Multiple opiate receptors in peripheral tissue preparations. *Biochemical Pharmacology* **30**, 1883–1887.

VON KÜGELGEN, I., ILLES, P., WOLF, D. & STARKE, K. (1985). Presynaptic inhibitory opioid δ- and κ-receptors in a branch of the rabbit ileocolic artery. *European Journal of Pharmacology* **118**, 97–105.