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DIFFERENT EFFECTS OF CURRENT STRENGTH ON INHIBITORY RESPONSES OF THE MOUSE VAS DEFERENS TO METHIONINE- AND LEUCINE-ENKEPHALIN

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The inhibitory potency of methionine (Met)-enkephalin on the field-stimulated mouse vas deferens was greatly increased by a reduction in current strength whilst that of leucine (Leu)-enkephalin increased only slightly. All currents were submaximal and all muscle twitches were neuronally evoked. These results suggest that inhibitory effects of Met- and Leu-enkephalin in the mouse vas deferens are not commonly mediated and provide a rapid method for ascertaining heterogeneity of enkephalin extracts.

Introduction In the original publication of the effects of opioids on the field-stimulated mouse vas deferens (Hughes, Kosterlitz & Leslie, 1975) it was noted that the strength of electrical stimulation influenced the ID_{50} values of opioid agonists. A reduction of current from 100 mA to 30 mA increased the inhibitory activity of normorphine 4 fold. Whilst using the mouse vas deferens for assay of enkephalin in brain extracts, we observed that a reduction in current strength increased the inhibitions produced by authentic Met-enkephalin to a greater degree than those produced by the extracts. Since these contain a mixture of Met- and Leu-enkephalin it was suspected that changes in current strength were not having an identical effect on the two peptides. We describe here a differential effect of current strength on Met- and Leu-enkephalin.

Methods Albino mice, T.O. strain, weighing 30 to 40 g were used in all experiments. Single vas deferens were mounted in a 3.5 ml bath in Mg^{2+}-free Krebs solution at 36°C, aerated with 95% O_2 and 5% CO_2 and maintained under a 200 mg tension. Field stimulation was provided by a constant current generator (designed at Chelsea College) linked to an SRI square wave stimulator. Rectangular pulses (1 ms, 0.1 Hz) were passed between linear platinum electrodes (0.3 mm dia × 6.5 cm length, 0.5 cm apart). Isometric contractions were recorded via a Grass FTO3 transducer linked to a Devices 3559 pre-amplifier and MX212 recorder.

Tissues were stimulated at 7 min intervals and dose-response curves for both peptides were obtained at four current strengths. ID_{50} values (molar concentration producing 50% inhibition of twitch) were calculated from linear regression of inhibitions between 20% and 80%; potency ratios at each current were calculated from paired experiments. Since ID_{50} values for agonists are normally distributed on a log scale (Fleming, Westfall, De la Lande & Jellett, 1972), values of Student's t were calculated from log ID_{50} and log potency ratios.

Results Increase in current strength produced an increase in twitch height of the mouse vas deferens which was submaximal up to 800 mA. The twitch height at 100 mA was about 30% of that at 800 mA. All these contractions were neurally evoked since they were abolished by tetrodotoxin (100 nM).

Met-enkephalin in common with other opioids (Hughes *et al.*, 1975) showed a marked increase in inhibitory activity as current strength was reduced. In contrast Leu-enkephalin showed only a small change in ID_{50} values as the current was reduced. This differential effect was reflected in the potency ratios from paired experiments in which Met- and Leu-enkephalin were almost equipotent at 100 mA, whilst at 800 mA Leu-enkephalin was 2.5 times more potent (Table 1). All dose-response curves were parallel but the characteristics of the inhibitory responses differed at high and low currents. The inhibition produced by Met-enkephalin was rapid in onset and well maintained at 100 mA whilst at 800 mA the recovery was rapid. Leu-enkephalin inhibitions were also rapid in onset but usually better maintained at all currents. There was no correlation between the tension developed by the stimulated muscle and the inhibition produced by the enkephalins. In a single experiment excluded from the data in Table 1 the Leu:Met potency ratio was extraordinarily high, being >5 at 500 mA and >7 at 800 mA.

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Discussion Recent evidence has indicated that opioids interact with multiple binding sites (Lord, Waterfield, Hughes & Kosterlitz, 1977). The differential effect of current strength indicates that the mediation of inhibitory responses in the mouse vas deferens is different for the two enkephalins. Ambache & Zar (1971) have provided evidence for the existence of two sets of postganglionic nerve fibres of different excitability in the guinea-pig vas deferens. Two components of the contractile response have also been observed in the mouse vas deferens (McGrath, 1978). One possible explanation for our results is that with higher current strengths there is an increase in the number and possibly types of intramural nerves stimulated. If this is so, then Leu-enkephalin, which had a similar ID_{50} at each of the currents examined, would be inhibiting all these nerves. Met-enkephalin, on the other hand, was more potent at 100 mA and its activity decreased as the current strength increased; this suggests that it inhibits mainly those nerves which are stimulated preferentially at low currents.

It is worthy of note that in a single experiment, the sensitivity of the tissue to Met-enkephalin was extremely low relative to Leu-enkephalin, indicative perhaps of a predominance of Leu-enkephalin-sensitive nerves. This in turn might be related to dissection of the tissue since the two components of contractile response in the vas vary from epididymal to prostatic ends (McGrath, 1978). With recent evidence of genetic variation in μ and δ receptors in the vas deferens of two strains of mice (Waterfield, Lord, Hughes & Kosterlitz, 1978) the possibility of a genetic abnormality in this isolated experiment cannot be ruled out.

A number of laboratories have used the mouse vas deferens for investigation of endogenous opioids and considerable variation in sensitivity to Met-enkephalin has been found. ID_{50} values of 4.2 nM (Rónai, Gráf, Székely, Dunai-Kovács & Bajusz, 1977), 12.8 nM (Waterfield, Smockum, Hughes & Kosterlitz, 1977) and 30 nM (Schulz, Wüster, Simantov, Snyder & Herz, 1977) are typical. This variability may in part reflect differences in the method of stimulation. This view is reinforced by the observation that with short trains of stimuli the ID_{50} value for Met-enkephalin is 67.9 nM (Shaw & Turnbull, 1978). Interestingly, their Leu:Met potency ratio of 3.63 is more than double that found by Waterfield *et al.* (1977), and higher than that observed in this study, which is consistent with our evidence that Leu-enkephalin is only slightly affected by alterations in the method of stimulation. The differential effects of current strength described here indicate the importance of maintaining constant stimulus parameters when using the mouse vas deferens for assaying mixtures of the enkephalins, and for the screening of analogues with reference to either of these peptides.

Preliminary experiments show that assays of mixtures, in which a single authentic peptide is used as standard, give different results at high and low currents and provide a rapid and reliable method for determining whether enkephalin samples are heterogenous. However, the potency ratios are too close to allow an accurate differential assay of the two peptides.

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Table 1 Effect of field stimulation current on ID_{50} values of Met- and Leu-enkephalin on the mouse vas deferens

	ID_{50} (nM)		
Current (mA)	*Met-enkephalin (Met)*	*Leu-enkephalin (Leu)*	*Potency ratio (Leu:Met)*
100	11.2 ± 1.5 (8)***	9.6 ± 1.5 (8)*	1.26 ± 0.19 (8)**
300	19.0 ± 3.7 (6)**	15.1 ± 3.4 (6) NS	1.38 ± 0.16 (6)*
500	28.4 ± 3.9 (6) NS	14.0 ± 1.9 (6) NS	1.94 ± 0.14 (5) NS
800	40.3 ± 5.1 (6)	17.2 ± 2.7 (5)	2.62 ± 0.36 (5)

ID_{50} values (mean ± s.e. mean) were calculated from linear regression of dose-response curves. Potency ratios were calculated from ID_{50} values of paired experiments. Number of observations in parentheses. *t* test (log ID_{50} values) versus 800 mA.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant.

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