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The in vitro pharmacology of D-Met^2,Pro^5-enkephalinamide

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D-Met^2,Pro^5-enkephalinamide (D-Met,Pro-EA, Bajusz et al 1977) is one of the most frequently studied synthetic enkephalin analogues (Morley 1980). We report the results of in vitro investigations carried out with it and with normorphine, β-endorphin and met-enkephalin as reference substances. The in vitro effects were determined in isolated organ preparations specifically sensitive to opioid effects, i.e. in electrically stimulated longitudinal muscle strip of guinea-pig ileum, cat nictitating membrane, mouse and rat vas deferens and rabbit ear artery preparations. The isolated organs were chosen such, that among them there is a preparation that is predictive for analgesia in the case of non-peptide opioid substances (guinea-pig ileum, Kosterlitz & Waterfield 1975), has high sensitivity to the opioid agonist (mouse vas deferens, Hughes et al 1975; Lord et al 1977; Rónai et al 1977) or the presynaptic inhibitory (rabbit ear artery, Knoll 1976; Rónai et al 1978) effect of met-enkephalin, or displays selectivity towards β-endorphin (rat vas deferens, Schulz et al 1979).

The experimental conditions for the electrically stimulated longitudinal muscle strip of guinea-pig ileum, cat nictitating membrane, mouse vas deferens and rabbit ear artery preparations were the same as those described by Rónai et al (1977) and Rónai & Berzétei (1978).

Rat vasa deferentia were prepared according to Schulz et al (1979). Stripped vasa taken from CFY rats (160–180 g) were set up in Mg^{2+}-free Krebs' solution containing 0·25 mM L-tyrosine, aerated with carbogen at 31 °C. The initial tension was 1·0 g. Low-frequency (0·1 Hz) field stimulation was applied; the parameters of individual pulses were: 1 ms impulse duration, 3 V cm^{-1} voltage-drop. The equilibration period was 120 min, under continuous stimulation.

The inhibitory potencies of compounds exerted upon the contractions/pressure changes elicited by electrical stimulation were characterized by the 50% inhibitory concentration (IC50) expressed uniformly in nanomol litre^{-1}. The antagonist effect of the opiate antagonist naltrexone determined against the different opioid compounds in the assay systems used, was characterized in terms of K_e values (Paton 1961; Kosterlitz & Watt 1968) also in nanomol litre^{-1}. In guinea-pig ileum the dose ratios were assessed by the single dose method (Kosterlitz & Watt 1968), whilst in the other preparations they were determined from dose-response curves of agonists taken in the absence and presence of antagonist (Arunlakshana & Schild 1959). The antagonist was left in contact with the organs for 20 min except for the nictitating membrane, where it was left for

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Table 1. The opiate agonist activities (IC50 values) of D-Met,Pro-EA and its reference substances in different isolated organs.

	IC50 values (n mol litre^{-1}) in				
Substances	GPI*	CNM	RVD	MVD	ART
D-Met,Pro-EA	19·7 ± 2·7 (n = 6)	36·9 ± 2·8 (n = 4)	1247 ± 197 (n = 33)	23·3 ± 1.3 (n = 12)	2300 ± 470 (n = 4)
Normorphine	187 ± 6 (n = 220)	717 ± 78 (n = 10)	>100 000 (n = 6)	248 ± 11 (n = 42)	>35 000 (n = 3)
β-endorphin	97 ± 7 (n = 12)	960 ± 32 (n = 3)	57 ± 9 (n = 8)	63 ± 4·4 (n = 40)	770 (n = 5)
Met-enkephalin	223 ± 26 (n = 17)	779 ± 160 (n = 14)	161 000 ± 29 000 (n = 6)	8·1 ± 0·4 (n = 115)	105 ± 9·3 (n = 4)

Mean ± s.e.m. values are given. Numbers of experiments are in parentheses.
* GPI = Ileum, CNM = nictitating membrane, RVD = rat vas deferens, MVD = mouse vas deferens, ART = rabbit ear artery.

Table 2. The K_e values of naltrexone in different isolated organs determined against opiate agonists of different character.

Substances	K_e† values (n mol litre^{-1}) of naltrexone in			
	GPI*	CNM	RVD	MVD
D-Met,Pro-EA	0·53 ± 0·06 (n = 4)	0·40 ± 0·09 (n = 4)	1·04 ± 0·11 (n = 13)	1·89 ± 0·20 (n = 8)
Normorphine	0·63 ± 0·08 (n = 4)	0·52 ± 0·14 (n = 6)	—	0·63 ± 0·11 (n = 7)
β-Endorphin	1·04 ± 0·15 (n = 6)	4·28 ± 0·58 (n = 4)	1·31 ± 0·23 (n = 7)	8·84 ± 0·64 (n = 6)
Met-enkephalin	0·81 ± 0·17 (n = 4)	4·04 ± 1·03 (n = 4)	+ +	7·14 ± 0·52 (n = 16)

* See Table 1 for key.
† K_e = equilibrium dissociation constant.
‡ Mean ± s.e.m. values are given, the Nos of experiments are in parentheses.
+ + = The inhibitory action of Met-enkephalin can be prevented or reversed specifically by naltrexone.
No kinetic analysis has been performed in ART; in this preparation only high ($5 \times 10^{-7} - 10^{-6}$ M) concentrations of naloxone/naltrexone were capable of preventing or reversing the inhibitory actions.

30 min. No kinetic analysis was performed for rabbit ear artery.

The results obtained with D-Met,Pro-EA and the reference substances are summarized in Tables 1 and 2. As it is apparent from Table 1, the in vitro activity pattern of D-Met,Pro-EA is quite distinct from that of met-enkephalin. In each isolated organ where β-endorphin exerted inhibitory action, D-Met,Pro-EA was also an effective inhibitor; however, the activity pattern of the enkephalin analogue failed to follow that of the untriakontapeptide. In this respect it was more like normorphine: in the isolated organs where the morphine-congener was practically ineffective (i.e. rat vas, rabbit artery), D-Met,Pro-EA also lost potency to a considerable extent compared with ileum and mouse vas deferens. The similarity between the actions of normorphine and D-Met,Pro-EA is supported further by their antagonism by naltrexone (Table 2).

Inspection of Tables 1 and 2 also leads to the conclusion that those results obtained in nictitating membrane and artery gave no additional information about the opioid properties of D-Met,Pro-EA, compared with the other preparations.

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