ACETALDEHYDE-ENKEPHALINS: PRONOUNCED CHANGES IN THE OPIATE ACTIVITY OF METHIONINE-ENKEPHALIN AND LEUCINE-ENKEPHALIN ON REACTION WITH ACETALDEHYDE

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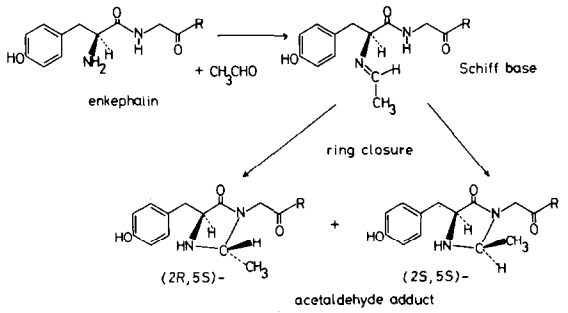
1. Introduction

We have shown that the two major endogenous opioid peptides, methionine-enkephalin and leucine-enkephalin [1,2] (enkephalin[Met^5] and enkephalin-[Leu^5]) rapidly react with acetaldehyde in aqueous buffers to give a stable 'acetaldehyde-enkephalin' adduct, referred to as 'acetaldehyde-enkephalin[Met^5]' and 'acetaldehyde-enkephalin[Leu^5]', respectively. The structure of these adducts was established as a diastereoisomeric mixture of the (2*R*, 5*S*)- and (2*S*, 5*S*)-2-methylimidazolidin-4-one derivatives of the parent peptides in which the acetaldehyde moiety forms a molecular bridge between the amide nitrogen of the Tyr$^{(1)}$–Gly$^{(2)}$ peptide bond and the α-NH_2 of the N-terminal Tyr residue [3]. This is shown in scheme 1. Here, we complement [3] by describing the influence that acetaldehyde adduct formation has on the intrinsic opiate activity of enkephalin[Met^5] and enkephalin[Leu^5].



Scheme 1

2. Materials and methods

Enkephalin[Met^5] and enkephalin[Leu^5] were obtained from Miles Labs. The corresponding acetaldehyde adducts were prepared as in [3]. [^{3}H]-Enkephalin[D-Ala^2, D-Leu^5] (52 Ci/mmol) was from the Radiochemical Centre, Amersham and [^{3}H]dihydromorphine (47 Ci/mmol) was from New England Nuclear Corp. Boston, MA. Bacitracin was obtained from Sigma Chemical Co. Naloxone hydrochloride was a gift from Endo Labs., NY and morphine sulphate was kindly provided by St Mary's Hospital Pharmacy.

2.1. *Opiate receptor binding assay*

Brain membranes from male Sprague-Dawley rats were prepared as in [4]. Aliquots of this preparation in 2.0 ml final vol. were incubated with either tritiated enkephalin (1.2 nm) or dihydromorphine (1.05 nm) and different concentrations of test substance at 0°C (ice/water mixture) for 2 h in the presence of bacitracin, 0.1 mg/ml. The incubation solutions were filtered on Whatman GF/B glass fibre filters, under vacuum, and washed with 2 X 4 ml ice-cold incubation buffer (Tris–HCl buffer, 50 mM, pH 7.4). Radioactivity was determined by scintillation counting using a Packard 3255 scintillation spectrometer. Non-specific binding was determined by incubations in the presence and absence of a large excess (10^{-6} M) of unlabelled enkephalin[Met^5] or naloxone. Assays were performed in triplicate at 6 or 7 concentrations of test substance, and the IC_{50} values obtained by graphical methods.

2.2. *Mouse vas deferens and guinea pig ileum*

Field stimulation of the isolated mouse vas def-

erens was done as in [5]. Test substances were added to the tissue preparation which was incubated in 2.0 ml Mg-free Krebs buffer solution and gassed with 5% CO_2 in O_2. Supramaximal coaxial stimulation of the guinea pig ileum [6,7] was done using a 30 ml organ bath gassed with 5% CO_2 in O_2 at 37°C. Respective tissues were stimulated using a Grass S-44 stimulator, and the resultant contractions transduced with a Grass FT-03 transducer. The transduced contractions were recorded with a Grass model 7C polygraph pen recorder. Dose–response curves were constructed from duplicate assays at 5 or 6 concentrations of each substance using at least 4 different tissue preparations. The results shown are the mean values obtained from these experiments.

3. Results and discussion

Whereas both enkephalin[Met^5] and enkephalin-[Leu^5] have potent agonist activity as shown by their ability to inhibit electrically evoked contractions of the isolated mouse vas deferens and the guinea pig ileum [1], the obverse is true for their respective acetaldehyde adducts (table 1; for comparison, data for morphine and N^{α}-ethyl-enkephalin[Met^5] are included). Enkephalin[Met^5] is ~300-times more active than its acetaldehyde adduct in the mouse vas deferens, and 100-times as potent in the ileum assay. Similarly, enkephalin[Leu^5] is ~550- and 70-times more active than its acetaldehyde derivative in the vas deferens and ileum, respectively. Therefore, in these two assay systems, acetaldehyde adduct formation causes a pronounced loss in activity when compared to the parent pentapeptides. For 'acetaldehyde-enkephalin[Met^5]' it appears that the observed fall in potency can be largely, but not exclusively, ascribed to alkylation of the α-NH_2 because N^{α}-ethylenkephalin-[Met^5] also displays greatly reduced activity. Presumably, ring formation also severely restricts the conformational mobility of the N-terminal region of the peptide; note [8,9] in which indicate that the relative flexibility of this region of the enkephalin molecule may be important in receptor binding.

The activity trend outlined above is similarly evinced by measuring opiate receptor binding in a particulate fraction from rat brain (table 2). Enkephalin[Met^5] is ~850-times and enkephalin[Leu^5] 300-times more potent than their acetaldehyde derivatives in inhibiting [^{3}H]enkephalin[D-Ala^2, D-Leu^5] binding. The inhibitory effect of the 'acetaldehyde-enkephalins' on [^{3}H]dihydromorphine binding is negligible. These results have been obtained with a diastereoisomeric mixture of the acetaldehyde adducts, and although the intrinsic activity of each mixture is very low it is conceivable that the individual isomers have differential activities depending on the opiate assay used; a solution to this particular conundrum must await the separation of each diastereoisomer. The binding data also show that acetaldehyde adduct formation leads to a loss of binding to both δ-receptors and μ-receptors [10–12].

The investigation of the intrinsic biological activity of these adducts is limited by their temperature-dependent decomposition to give the parent peptide and acetaldehyde [4]. Because 'acetaldehyde-enkephalins' are markedly less active than their precursor pentapeptides any fractional decomposition will give abnormally high apparent biological potencies. Therefore, each investigation should begin with newly syn-

Table 1
Inhibition of electrically induced contractions of the isolated mouse vas deferens and the guinea pig ileum by morphine and enkephalin derivatives

Test substance	IC_{50} (morphine) / IC_{50} (peptide)	
	Vas deferens	Ileum
Morphine[a]	1.0	1.0
Enkephalin[Met^5]	35.70	0.75
Acetaldehyde-enkephalin[Met^5]	0.12	0.008
Enkephalin[Leu^5]	39.20	0.24
Acetaldehyde-enkephalin[Leu^5]	0.07	0.0036
N^{α}-Ethylenkephalin[Met^5]	0.31	0.05

[a] The mean IC_{50} value for morphine was 500 and 73 nm for the mouse vas deferens and the guinea pig ileum, respectively

Table 2
Comparison of the displacement of [^{3}H]enkephalin[D-Ala^2, D-Leu^5] and [^{3}H]dihydromorphine binding from brain membranes by morphine and enkephalin derivatives

Unlabelled compound	IC_{50} (morphine) / IC_{50} (peptide)	
	[^{3}H]-enkephalin-[D-Ala^2, D-Leu^5]	[^{3}H]-dihydro-morphine
Morphine[a]	1.0	1.0
Enkephalin[Met^5]	10.4	0.58
Acetaldehyde-enkephalin[Met^5]	0.012	0.0005
Enkephalin[Leu^5]	5.5	0.54
Acetaldehyde-enkephalin[Leu^5]	0.018	0.0005
N^{α}-Ethylenkephalin[Met^5]	0.92	0.0006

[a] The mean IC_{50} value for morphine was 46 and 2.9 nm for the inhibition of [^{3}H]-enkephalin[D-Ala^2, D-Leu^5] and [^{3}H]dihydromorphine binding, respectively

thesised material; the consequences of acetaldehyde adduct breakdown should be considered in all further studies.

Chemical studies indicate that the critical amino acid sequence necessary for rapid acetaldehyde adduct formation is Tyr–Gly–Gly– and this also holds for the largest opioid peptide β-endorphin, which similarly reacts with acetaldehyde and undergoes a change in opiate activity [4]. It seems very likely that all the larger opioid peptides with the enkephalin[Met^5] N-terminal sequence [13,14] and α-neo-endorphin [15] and dynorphin-(1–13) [16] will also form stable acetaldehyde adducts with concomitant changes in opiate activity.

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