SYNDYPHALIN SD-25: A HIGLY SELECTIVE LIGAND FOR μ OPIATE RECEPTORS

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1. Introduction

After the discovery of the enkephalin [1], it was reported [2] that the order of potency for morphine and the enkephalins was inversed in the mouse vas deferens (in comparison with the guinea pig ileum), thus suggesting the existence of various class of opiate receptors. However, a major problem in the precise characterization of the different receptors has been the absence of very selective ligands for one peculiar subclass of opiate receptors. In [3] morphiceptin, the amide of a fragment of the milk protein β-casein, was suggested to represent a highly selective ligand for the μ opiate receptor. In [4] the enkephalin analogue, Tyr–D-Ser– Gly–Phe–Leu–Thr was proposed as a selective ligand for the δ opiate receptor. Synthesis of various enkephalin analogues with potent activity in the guinea pig ileum bioassay as well as significant analgesic activity after subcutaneous administration was reported in [5–7]. One of these analogues, Tyr–D-Met–Gly–MePheol, named 'syndyphalin (SD)-25' was 23 300-times and 9-times as active as morphine in the guinea pig ileum bioassay and in the tail flick analgesic test after subcutaneous administration, respectively [7]. Here, we report the potency of this analogue on the μ, δ and κ opiate receptors using [^{3}H]dihydromorphine (DHM), D-[^{3}H]Ala^2,D-Leu^5-enkephalin (DADLEU) and [^{3}H]ethylketocyclazocine (EKC) as their respective ligands. Our results show that SD-25 is a highly specific and selective ligand for the μ opiate receptor.

2. Materials and methods

Binding experiments were performed using frozen slide-mounted brain sections as in [8] for [^{3}H]DHM and [^{3}H]DADLEU and as in [9,10] for [^{3}H]EKC. Briefly, male Sprague-Dawley rats (~ 200 g body wt) were sacrificed by decapitation. Their brains were rapidly excised, quick-frozen in isopentane at –40°C and mounted on cryostat chucks. The cortex was carefully shaved off down to the level of the corpus callosum, and 25 μn-thick coronal sections of caudate-putamen at midstriatal levels were cut at –14°C. Sections were thaw-mounted near the edge of cleaned gelatin-coated slides, air-dried on ice for ~ 2 h, and then dried at –14°C for at least 48 h before use. Slide mounted sections (1 section/slide) were incubated in 30 ml beakers in 10 ml as follows: for [^{3}H]DHM, in a 50 mM Tris–HCl buffer containing 3 mM Mn $(OAc)_2$, 1% BSA, 1 nM [^{3}H]DHM (65 Ci/mmol; Amersham) at pH 7.4, 25°C for 30 min with various concentrations of SD-25, morphiceptin (Peninsula) or other opiates; for [^{3}H]DADLEU, in a 50 mM Tris–HCl buffer containing 100 mM NaCl, 3 mM Mn $(OAc)_2$, 2 μM GTP, 1% BSA, 2.5 nM [^{3}H]DADLEU (25 Ci/mmol; Amersham) at pH 7.4, 25°C for 30 min with various concentrations of SD-25, morphiceptin or other opiates; for [^{3}H]EKC, in a 50 mM K_2HPO_4 · HCl buffer containing 1 mM EDTA, 100 mM NaCl, 2.3 nM [^{3}H]EKC (15 Ci/mmol; New England Nuclear) at pH 7.4, 4°C for 60 min with various concentrations of SD-25, morphiceptin or other opiates. At the end of the incubation, the slides were placed in racks holding 30 slides and transferred sequentially through 6 rinses (20 s in each) of incubation buffer without salt (pH 7.4) at 4°C plus 1% BSA. Binding of [^{3}H]-labeled ligands to the tissue slice was quantitated by counting the tissue-laden slide fragment in 10 ml Aquassure scintillation cocktail (New England Nuclear) after vigorously agitating the vial contents for 30 min. Specific binding was calculated as the difference in counts bound in the presence and absence of 1 μM

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etorphine for [^{3}H]DHM and [^{3}H]DADLEU and 10 μM ECK for [^{3}H]EKC. Synthesis of syndyphalin (SD)-25 was described in [7].

3. Results and discussion

SD-25 is more potent than morphine itself in displacing [^{3}H]DHM binding, a prototype μ opiate receptor ligand (fig.1). SD-25 appears to possess much more affinity for this site than morphiceptin. In fact, SD-25 is probably the most potent peptide or peptide analogue on μ opiate binding site [11].

The high selectivity of SD-25 is very interesting. SD-25 is a very weak displacer of [^{3}H]DADLEU and [^{3}H]EKC, respectively prototypes of the δ and κ opiate binding sites (fig.2,3). These results indicate that SD-25 is a very selective ligand for the μ opiate binding, being at least as selective as morphiceptin (table 1) [3]. Subsequent studies using SD-25 as a highly selective ligand may reveal more precisely the structural requirement of this opiate receptor subtype. Moreover, conformational analysis of this peptide analogue should help to design an even more selective ligand.

Based on our binding assay data, the potency of SD-25 in the guinea pig ileum would have been expected to be lower than found (table 1) [7], suggesting that the great potency of SD-25 in this bioassay is not only related to an increased affinity for the binding site, but also to other factors such as an increased resistance to enzymatic degradation. The greater analgesic potency of SD-25 compared to mor-

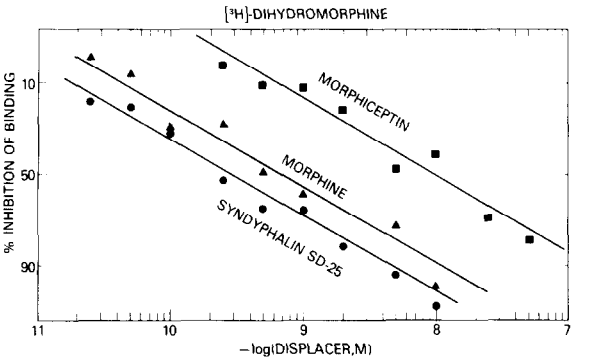

Fig.1. Displacement of [^{3}H]DHM by SD-25, morphiceptin and morphine. Slide-mounted caudate sections were incubated with 1.0 nM [^{3}H]DHM and increasing amounts of the various opiates under study. Non-specific binding in presence of 1.0 μM etorphine has been subtracted from all experimental points. Values are expressed as percentage inhibition of specific binding of [^{3}H]DHM and are shown as log-probit plots. The experiment has been replicated 3-times and varied <15%.

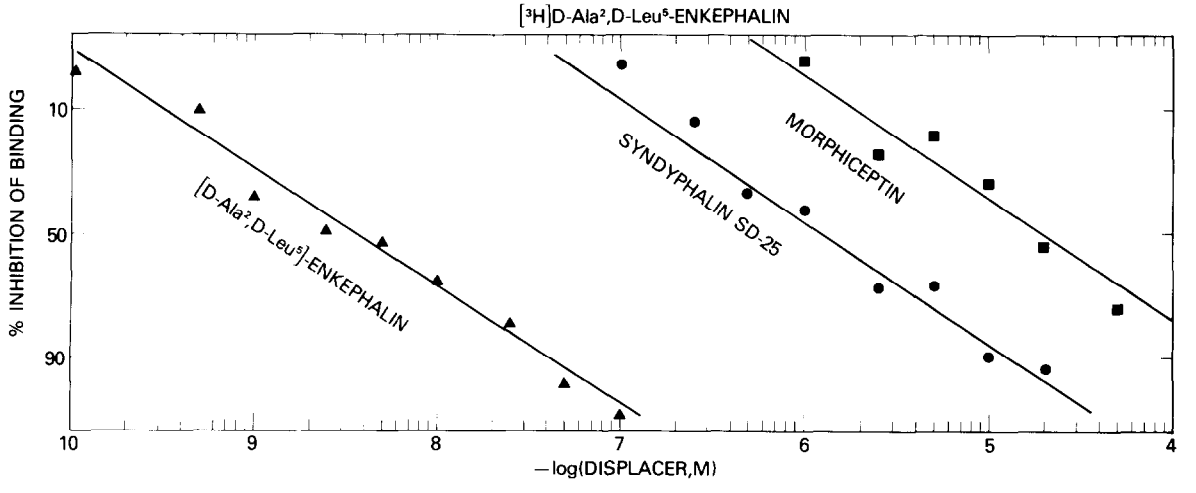

Fig.2. Displacement of [^{3}H]DADLEU by SD-25, morphiceptin and DADLEU. Slide-mounted caudate sections were incubated with 2.5 nM [^{3}H]DADLEU and increasing amounts of the various opiates under study. Non-specific binding in presence of 1.0 μM etorphine has been subtracted from all experimental points. Values are expressed as percentage inhibition of specific binding of [^{3}H]DADLEU and are shown as log-probit plots. The experiment has been replicated 3-times and varied <15%.

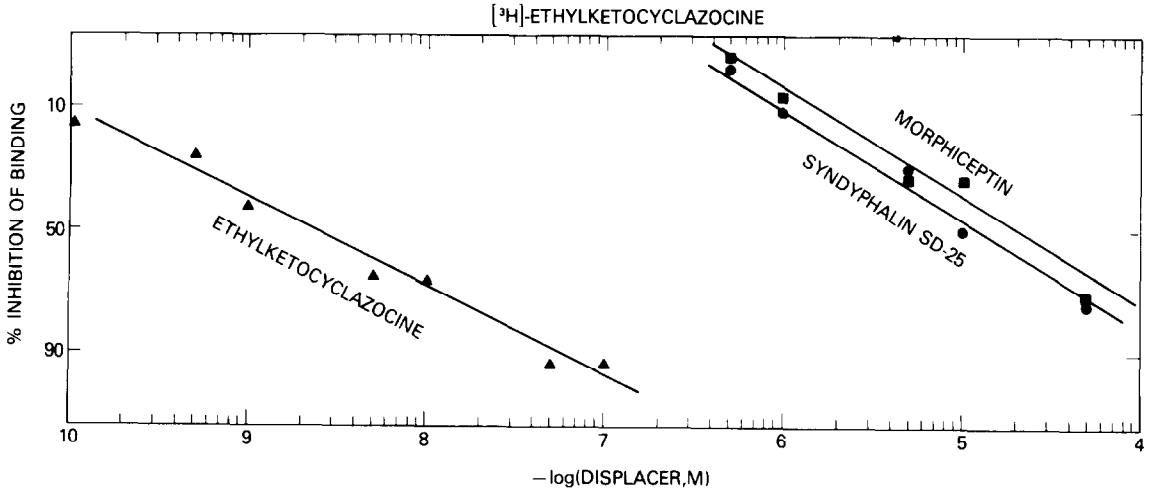

Fig.3. Displacement of [³H]EKC by SD-25, morphiceptin and EKC. Slide-mounted caudate sections were incubated with 2.3 nM [³H]EKC and increasing amounts of the various opiates under study. Non-specific binding in presence of 10 μM EKC has been subtracted from all experimental points. Values are expressed as percentage inhibition of specific binding of [³H]EKC and are shown as log-probit plots. The experiments has been replicated 3-times and varied <20%.

phine (table 1) [7] might be due to a combination of these factors namely the high affinity for the μ opiate binding site, an easier crossing of the blood–brain barrier, and the resistance to enzymatic degradation. Our results also support the hypothesis that μ opiate receptors mediate the analgesic effects of opiates [12], since SD-25 is a potent analgesic devoided of affinity for δ and κ opiate receptors. Finally, the very high analgesic effect of SD-25 after subcutaneous administration may indicate that the development of a long-lasting, orally effective opiate peptide analgesic might be expected in the near future.

Table 1
Potency of syndyphalin SD-25 and morphiceptin on various binding sites and in some biological systems

Compound	[³H]DHM IC_{50}[a] (nM)		[³H]DADLEU IC_{50} (nM)		[³H]EKC IC_{50} (nM)		Guinea pig ileum ED_{50}[b] (nM)		Analgesic effect	
									Tail flick ED_{50}[c] (mg/kg, s.c.)	Writhing
Syndyphalin SD-25	0.29	0.05	1250	200	13 000	3000	0.0025	0.0009[c]	0.54[c]	0.15[c]
Morphiceptin	10.0	1.6	19 500	3000	21 000	4000	130[d]		–	–
Morphine	0.78	0.1	–		–		52.4	6.3[c]	2.55[c]	0.50[c]
DADLEU	–		3.4	0.6	–		–		–	–
EKC	–		–		2.4	0.5	–		–	–

[a] IC_{50} is the concentration which inhibits the binding of the labeled ligand by 50%; values represent mean ± SEM of 3 determinations, each in triplicate

[b] ED_{50} is the concentration which suppresses the electrically stimulated muscle contraction by 50% or the dose which induces analgesia in 50% of the animals

[c] Data from [7]

[d] Data from [3]

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