SYNDYPHALIN-33, A SYNTHETIC TRIPEPTIDE ALKYLAMIDE WITH PROLONGED ANALGESIC ACTIVITY

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1. Introduction

Pentapeptides, Met-enkephalin (ENK) and Leu-ENK [1] with opiate agonist activity produce only weak and short-lived analgesia even after intracerebroventricular (i.c.v.) administration [2,3] and no analgesia follows their subcutaneous (s.c.) administration. This is due to their rapid enzymatic degradation, and various attempts have been made to obtain ENK derivatives with high and prolonged analgesic activity by protecting the N- and C-terminals and the peptide bond of the molecule [4].

In [5–7], we reported that [MeTyr^1,Metol^5]-ENK (SD-6) and [MeTyr^1,D-Ala^2,Metol^5]-ENK (SD-14) have high and prolonged opioid activity in vitro and analgesia after i.c.v. administration. SD-6, however, produces only weak analgesia after s.c. administration [8]. This discrepancy might be due to its difficulty to cross the blood–brain barrier and/or its rapid degradation by endopeptidases [4].

Short-chain ENK analogues Tyr–D-Ala–Gly–alkylamides and Tyr–D-Ala–Gly–Pheol (SD-13) have opioid activities in vitro but do not show any significant analgesia after systemic administration [6–11]. Tyr–D-Met(O)–Gly–Pheol (SD-20) and Tyr–D-Met(O)–Gly–MePheol (SD-25) exhibit high and prolonged analgesia after s.c. administration [11], and the acetylated derivatives of the hydroxyl group in SD-20 also produce analgesia after s.c. administration [8]. These findings indicate that all 5 amino acids in ENK are not necessary for s.c. analgesic activity.

We therefore sought the 'minimal segment' of ENK required for s.c. analgesia and could find a tripeptide alkylamide with s.c. analgesia [12]. Here, we report the synthesis of a simple tripeptide Tyr–D-Met(O)–Gly–MPA named 'syndyphalin (SD)-33' (fig.1) which exhibits potent opioid activity in vitro

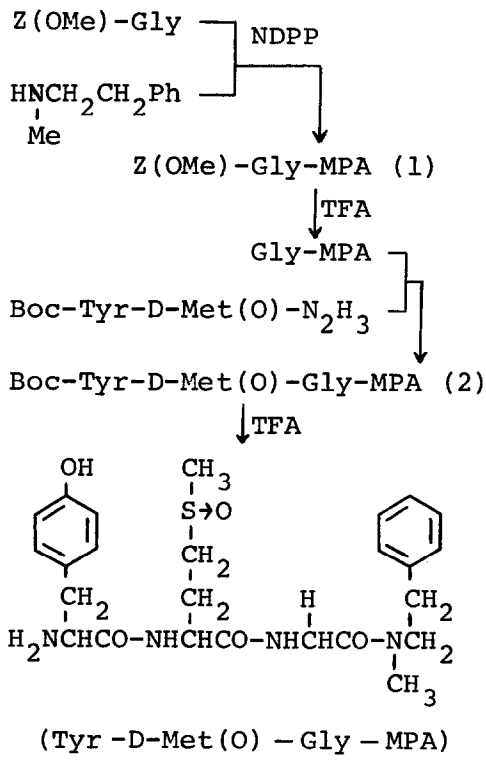

Fig.1. Synthetic scheme and structural formula of SD-33.

Abbreviations: MeTyr, *N*-methyl-L-tyrosine; Metol, L-methioninol; Pheol, L-phenylalaninol; D-Met(O), D-methionine sulfoxide; MePheol, *N*-methyl-L-phenylalaninol; MPA, *N*-methylphenethylamide; PA, phenethylamide; Z(OMe), *p*-methoxybenzyloxycarbonyl; NDPP, norborn-5-ene-2,3-dicarboximido diphenyl phosphate; TFA, trifluoroacetic acid; Boc, *t*-butoxycarbonyl

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and analgesia after s.c. administration. SD-33 is the only tripeptide alkylamide exhibiting substantial analgesia after s.c. administration.

2. Experimental

2.1. *Syntheses of peptides*

Tripeptide alkylamides were chemically synthesized as in [5,6,11]. Thin-layer chromatograms were run on Merck silica gel 60 F-254 pre-coated plates (0.25 mm). $R_F{}^1$ and $R_F{}^2$ values refer to the systems of chloroform–methanol–water (8:3:1, lower layer) and *n*-butanol–acetic acid–water (3:1:1), respectively.

2.1.1. Synthesis of Tyr–D-Met(O)–Gly–MPA (SD-33)

The synthetic scheme is outlined in fig.1. Z(OMe)–Gly and *N*-methylphenethylamine were condensed using NDPP [13] in the presence of triethylamine in ethyl acetate. After the coupling reaction for 24 h at room temperature, Z(OMe)–Gly–MPA (**1**) was obtained in 93% yield (m.p. 77–78°C; $R_F{}^1$ 0.85; satisfactory elemental analysis for $C_{20}H_{24}O_4N_2$).

Compound **1** was deblocked with TFA–anisole, and the resulting Gly–MPA was condensed with Boc–Tyr–D-Met(O)–N_2H_3 [11] by the azide method in dimethylformamide. After the coupling reaction for 48 h at 4°C, Boc–Tyr–D-Met(O)–Gly–MPA (**2**) was obtained in 69% yield (m.p. 116–118°C; $R_F{}^1$ 0.79; satisfactory analysis for $C_{30}H_{42}O_7N_4S \cdot 1.5\ H_2O$).

Compound **2** was deblocked with TFA–anisole, and the deblocked material was converted to the acetate form by the treatment with Amberlite IRA-400 (acetate cycle). This solid material was reprecipitated from methanol–ethyl acetate, and Tyr–D-Met(O)–

Table 1
Opioid activity on guinea pig ileum and analgesic activities in mice after s.c. administration

Compound	Effect on the isolated guinea pig ileum		
	ED_{50} [M] (mean ± SEM)		Relative potency[a]
Morphine · HCl	$5.24 \pm 0.63 \times 10^{-8}$	(n = 37)[b]	1.00
Tyr–Gly–Gly–Phe–Met (Met–ENK)	$3.35 \pm 1.17 \times 10^{-8}$	(n = 21)	1.56
Tyr–D-Met(O)–Gly–PA (SD-32)	$6.16 \pm 1.49 \times 10^{-9}$	(n = 6)	8.51
Tyr–D-Met(O)–Gly–MPA (SD-33)	$4.66 \pm 1.53 \times 10^{-9}$	(n = 6)	11.24
	Analgesic activity in the tail flick test		
	ED_{50} [mg/kg, s.c.] (95% confidence limits)		Relative potency[a]
Morphine · HCl	2.55 (1.81–3.58)	(n = 30)	1.00
Tyr–Gly–Gly–Phe–Met (Met–ENK)	>80	(n = 5)	–
Tyr–D-Met(O)–Gly–PA (SD-32)	>80	(n = 25)	–
Tyr–D-Met(O)–Gly–MPA (SD-33)	4.04 (2.67–7.21)	(n = 39)	1.14
	Analgesic activity in the writhing test		
	ED_{50} [mg/kg, s.c.] (95% confidence limits)		Relative potency[a]
Morphine · HCl	0.500 (0.315–0.794)	(n = 18)	1.00
Tyr–Gly–Gly–Phe–Met (Met–ENK)	>25	(n = 5)	–
Tyr–D-Met(O)–Gly–PA (SD-32)	≧10	(n = 20)	0.09≧
Tyr–D-Met(O)–Gly–MPA (SD-33)	0.801 (0.374–1.72)	(n = 25)	1.13

[a] Relative potency is on a molar basis (morphine = 1.00)
[b] n = number of experiments or mice used

Gly–MPA (SD-33) was obtained as the acetate form in 89% yield (m.p. 140–144°C; R_F^1 0.25, R_F^2 0.32; satisfactory analysis for $C_{25}H_{34}O_5N_4S \cdot CH_3CO_2H \cdot H_2O$).

2.1.2. Synthesis of Tyr–D-Met(O)–Gly–PA (SD-32)

SD-32 was synthesized by the same method as SD-33. Z(OMe)–Gly–PA was obtained from Z(OMe)–Gly and phenethylamine by condensing with NDPP in 85% yield (m.p. 124–125°C; R_F^1 0.93; satisfactory analysis for $C_{19}H_{22}O_4N_2$).

Boc–Tyr–D-Met(O)–Gly–PA was obtained from Boc–Tyr–D-Met(O)–N_2H_3 and Gly–PA (deblocked from Z(OMe)–Gly–PA with TFA–anisole) by the azide method in 78% yield (m.p. 112–115°C; R_F^1 0.74; satisfactory analysis for $C_{29}H_{40}O_7N_4S \cdot H_2O$).

Tyr–D-Met(O)–Gly–PA (SD-32) was obtained from the protected tripeptide amide by deblocking procedure with TFA as the acetate form in 89% yield (m.p. 102–104°C; R_F^1 0.22, R_F^2 0.34; satisfactory analysis for $C_{24}H_{32}O_5N_4S \cdot CH_3CO_2H \cdot 1.5\ H_2O$).

2.2. *Bioassay*

Biological activities were examined as in [11]. The opioid activity was determined in vitro by inhibition of electrically evoked contraction of the isolated guinea pig ileum [14]. Analgesia following s.c. administration was estimated in the tail flick [15,16] and phenylquinone writhing [16,17] tests in mice.

3. Results and discussion

The in vitro and in vivo opiate agonist activities are summarized in table 1. Both Tyr–D-Met(O)–Gly–PA (SD-32) and SD-33 showed a potent in vitro opioid activity in the guinea pig ileum assay. Their activities were reversed by naloxone.

In the tail-flick test after s.c. administration, SD-32 was <5% as potent as morphine, but *N*-methylation of phenethylamide caused a remarkable increase in potency; SD-33 exhibited analgesic activity nearly comparable to that of morphine. A similar

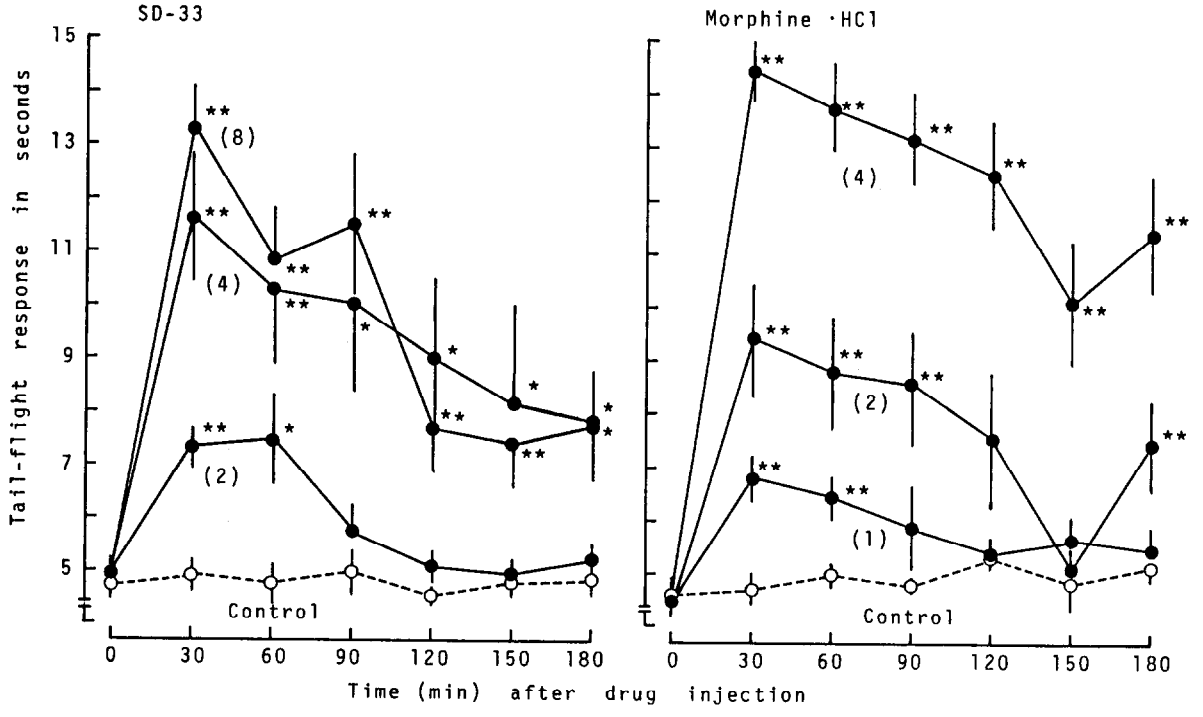

Fig.2. Time-course of analgesic activity of SD-33 and morphine in the tail flick test in mice: ( ) dose in mg/kg, sub-cutaneously; * $0.01 < P < 0.05$; ** $P < 0.01$ different from each pre-drug value. Each point and vertical bar represent the mean and SEM from 10 mice.

phenomenon was observed for potential increase in the case of SD-25 which was obtained by the *N*-methylation of phenylalaninol in SD-20 [11]. The analgesic effect of SD-33 was relatively durable; ED_{50} values were 4.15, 7.02 and 5.44 mg/kg (n = 30) at 0.5, 1 and 1.5 h, respectively, after s.c. administration, and those of morphine were 2.9, 2.7 and 4.6 mg/kg (n = 30) at 0.5, 1 and 1.5 h, respectively (fig.2). The effect of SD-33, like that of morphine, was reversed by naloxone. It was surprising that a simple tripeptide alkylamide, such as SD-33, exhibited such a potent analgesic activity after s.c. administration.

In the writhing test after s.c. administration, SD-33 and SD-32 were in agreement with the results in the tail-flick test. The potency of SD-33 was nearly comparable to that of morphine, while SD-32 was <10% as potent as morphine.

For the first time, a tripeptide alkylamide has been prepared which exhibits substantial analgesia after s.c. administration and SD-33 may be the 'minimal segment' of an opioid peptide required for s.c. analgesia, although structurally it bears little resemblance any longer to enkephalin. The stability in vitro and in vivo, the ready availability and high melting point (140–144°C) should make it valuable for further studies on opioid peptides.

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