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DALDA analogues containing α-hydroxymethylamino acids★✪

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To evaluate the role of aromatic amino-acids residues, four analogues of the μ-selective opioid peptide agonist DALDA (H-Tyr-D-Arg-Phe-Lys-NH_2) containing the amphiphilic, α,α-disubstituted amino acid (*R*)- or (*S*)-α-hydroxymethyltyrosine (HmTyr) in position 1 and (*R*)- or (*S*)-α-hydroxymethylphenylalanine (HmPhe) in position 3 of the peptide sequence were synthesized. Only the [(R)-HmPhe^3)]DALDA analogue displayed full agonistic activity in both the guinea pig ileum and the mouse vas deferens assays and turned out to be a δ receptor-selective opioid agonist.

Centrally acting μ opioid agonists are still the most frequently used analgesics for the relief of severe pain, but their usefulness is limited by a number of well known side-effects, including tolerance, physical dependence, respiratory depression, adverse gastrointestinal effects, etc. For that reason, current efforts to develop centrally acting opioid analgesics are directed toward developing compounds with different opioid activity profiles (e.g. δ ago-

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Abbreviations: Boc, *tert*-butyloxycarbonyl; Cl-Z, *p*-chlorobenzyloxycarbonyl; DALDA, H-Tyr-D-Arg-Phe-Lys-NH_2; DAMGO, H-Tyr-D-Ala-Gly-N$^{\alpha}$MePhe-Gly-ol; DIEA, diisopropylethylamine; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; GPI, guinea pig ileum; HOBt, 1-hydroxybenzotriazole; MVD, mouse vas deferens; TBTU, O-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid.

nists or mixed μ agonist/δ antagonists) [1]. Opioid peptides with high μ receptor binding affinity and high μ receptor selectivity include several enkephalin analogues as well as morphiceptin and its analogues [2].

Among various analogues of the N-terminal tetrapeptide segment of dermorphin, TAPS (H-Tyr-D-Arg-Phe-Ser-OH) [3] and DALDA (H-Tyr-D-Arg-Phe-Lys-NH_2) have been reported to be potent and selective μ agonists [4].

In the present paper we describe four analogues of DALDA containing the amphiphilic, α,α-disubstituted amino acid: (*R*)- or (*S*)-α-hydroxymethyltyrosine (HmTyr) in position 1 and (*R*)- or (*S*)-α-hydroxymethylphenylalanine (HmPhe) in position 3 of the peptide.

MATERIALS AND METHODS

Chemistry. Racemic α-hydroxymethyltyrosine and α-hydroxymethylphenylalanine were synthesized by selective α-hydroxymethylation [5, 6] and were resolved into the enantiomers by fractional crystallization of the diastereomeric salts of their N-benzoyl derivatives with (–)-quinine, using the method described in the literature [7, 8]. (*R*)- and (*S*)-Boc-HmTyr as well as (*R*)- and (*S*)-Boc-HmPhe were prepared as described elsewhere [9].

The peptide analogues were synthesized by the manual solid-phase method using a 4-methylbenzhydrylamine resin × HCl (100–200 mesh, 0.80 mmole/g, (Novabiochem)). N^{α}-Boc-protected amino acids were obtained from commercial sources. Starting with 0.25 g (0.2 mmole) of resin the following protected amino acids were added in a stepwise fashion to the growing peptide chain: N^{α}-BocLys(Cl-Z), N^{α}-Boc-Phe, N^{α}-Boc-D-Arg-(Tos) and N^{α}-(*R*)-Boc-HmTyr or N^{α}-(*S*)-Boc-HmTyr. Amino acids were coupled in a 3-fold excess using TBTU (3 equiv.), HOBt (3 equiv.) in the presence of DIEA (6 equiv.). For "difficult" couplings, when an α-hydroxymethyl-amino acid was acylated or used as an acylating component, prolonged reaction times (8 and 16 h for repeated coupling) were necessary. Removal of the Boc protecting group was performed by treatment with 50% (v/v) TFA in CH_2Cl_2 for 5 and 20 min, followed by CH_2Cl_2 washes (3 × 1 min), neutralization with 10% DIEA in CH_2Cl_2 for 5 and 10 min and CH_2Cl_2 washes (3 × 1 min). Completeness of the coupling reactions was monitored with the ninhydrin test [10]. The peptides were cleaved from the resin with anhydrous HF (5 ml/g resin) with anisole added as scavenger (1 ml/g resin) for 1 h at 0°C. After evaporation of the HF, the resin was extracted three times with anhydrous ethyl ether and subsequently with a 50% aqueous solution of acetic acid. The crude peptides were obtained in solid form by lyophilization of the acetic acid extracts and were purified by preparative reversed-phase HPLC on a Vydac C_{18} column (25 × 2.2 cm) with a linear gradient of 5–25% B at a flow rate of 12 ml/min. Each peptide was >98% pure as determined by analytical reversed-phase HPLC on a Vydac C_{18} column (25 × 0.46 cm) using a linear gradient of 10–20% B over 25 min at a flow rate of 1 ml/min, with UV detection at 220 nm. Solvents: (A) 0.05% trifluoroacetic acid in water and (B) 0.038% trifluoroacetic acid in acetonitrile/H_2O (90 : 10, v/v). Relative molecular masses were confirmed by FAB-MS (642.40 (M+H), 664.34 (M+Na), calc. for $C_{31}H_{47}O_6N_9$ 641.75). Analytical data of all the DALDA analogues synthesized are presented in Table 1.

Biology. For the determination of their *in vitro* opioid activities, the synthesized DALDA analogues were tested in bioassays based on the inhibition of electrically evoked contractions of guinea pig ileum (GPI) and mouse vas deferens (MVD). The GPI assay is usually considered as being representative for μ opioid receptor interactions, even though the ileum does also contain κ opioid receptors. In the MVD assay opioid effects are primarily mediated by δ opioid receptors but μ and κ recep-

Table 1. Analytical parameters of DALDA analogues

Compound	TLC		HPLC[c] t_R	FAB-MS $(M+H)^+$
	I[a]	II[b]		
1. (*R*)-HmTyr-D-Arg-Phe-Lys-NH_2	0.28	0.09	10.20	642
2. (*S*)-HmTyr-D-Arg-Phe-Lys-NH_2	0.28	0.09	10.57	642
3. Tyr-D-Arg-(*R*)-HmPhe-Lys-NH_2	0.52	0.29	12.15	642
4. Tyr-D-Arg-(*S*)-HmPhe-Lys-NH_2	0.52	0.29	12.83	642

[a]1-Butanol/acetic acid/ethyl acetate/water (1:1:1:1, by vol.); [b]1-butanol/acetic acid/water (4:1:1, by vol.); [c]Linear gradient of 10–20% B over 25 min at a flow rate of 1 ml/min.

tors also exist in this tissue. The GPI and MVD bioassays were carried out as reported in details elsewhere [11, 12].

potent δ agonist in the MVD assay. The MVD/GPI IC_{50} ratio of this compound is 0.373, in contrast to the ratio of 3.07 deter-

Table 2. Guinea pig ileum (GPI) and mouse vas deferens (MVD) assay of opioid peptide analogues[a]

Compound	GPI IC_{50} [nM]	MVD IC_{50} [nM]	MVD/GPI IC_{50} ratio
1. (*R*)-HmTyr-D-Arg-Phe-Lys-NH_2	>10 000 (P.A. 9%)[b]	>10 000 (P.A. 25%)[a]	–
2. (*S*)-HmTyr-D-Arg-Phe-Lys-NH_2	>10 000 (P.A. 14%)[a]	>10 000 (P.A. 33%)[a]	–
3. Tyr-D-Arg-(*R*)-HmPhe-Lys-NH_2	5310 ± 470	1980 ± 230	0.373
4. Tyr-D-Arg-(*S*)-HmPhe-Lys-NH_2	>10 000 (P.A. 13%)[a]	>10 000 (P.A. 21%)[a]	–
5. Tyr-D-Arg-Phe-Lys-NH_2 (DALDA)	254 ± 27	781± 146	3.07

[a]Mean of three determinations ± S.E.M.; [b]P.A., partial agonists (value in parentheses indicates maximal inhibition of contractions (%) obtained at a concentration of 10 μM)

RESULTS AND DISCUSSION

All four DALDA analogues synthesized were examined in the GPI and MVD bioassays. The results are presented in Table 2. As can be seen, the two analogues containing (*R*)- and (*S*)-α-hydroxymethyltyrosine in position 1 of the peptide are essentially inactive, but do show some weak partial agonist activity at a very high concentration (10 μM) in both cases. The analogue containing (*R*)-α-hydroxymethylphenylalanine in position 3 was a full agonist in both assays, albeit with a lower potency than the DALDA parent peptide. In the GPI assay it showed about 20-fold lower μ agonist potency than DALDA itself. Interestingly, however, it turned out to be only a 2.5-fold less mined for the DALDA parent peptide (Table 2). This result indicates that introduction of the hydroxymethyl group at the Phe^3 residue converted the μ-selective DALDA parent peptide into a δ-selective agonist. The analogue containing (*S*)-α-hydroxymethylphenylalanine in position 3 was almost inactive, showing only weak partial agonism at a high concentration in both assays.

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