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Receptor constants for endomorphin-1 and endomorphin-1-ol indicate differences in efficacy and receptor occupancy

Mahmoud Al-Khrasani [a], György Orosz [b], László Kocsis [b], Viktor Farkas [b], Anna Magyar [b], Imre Lengyel [c], Sándor Benyhe [c], Anna Borsodi [c], András Z. Rónai [a,*]

[a] *Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University Budapest, P.O.B. 370, H-1445, Budapest, Hungary*
[b] *Research Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös University Budapest, Budapest, Hungary*
[c] *Biological Research Center of Hungarian Academy of Sciences, Szeged, Hungary*

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Abstract

The opioid properties of endomorphin derivatives containing a C-terminal alcoholic(-ol) function were compared to the parent amidated compounds in isolated organs (longitudinal muscle strip of guinea-pig ileum and mouse vas deferens). Similar data were also generated for the μ-opioid receptor selective agonist synthetic peptide (D-Ala^2, MePhe^4, Gly^5-ol)-enkephalin (DAMGO) and its Gly^5-NH$_2$ congener (DAMGA). Endomorphin-1-ol (Tyr-Pro-Trp-Phe-ol) had an IC_{50} of 80.6 nM in mouse vas deferens and 61.2 nM in guinea-pig ileum; the corresponding values for endomorphin-2-ol (Tyr-Pro-Phe-Phe-ol) were 49.6 and 48.2 nM, for DAMGO 59.8 and 29.2 nM, respectively. As it was indicated by the antagonism by naltrexone, the agonist actions were exerted exclusively at μ-opioid receptors in both organs. The -ol derivatives were slightly (2.3–4.3 times) less potent than the parent amides in the bioassays: all peptides had, apparently, full agonist properties in intact preparations. With the aim of revealing potential partial agonist properties among the investigated peptides, we partially inactivated the μ-opioid receptor pool in mouse vas deferens by 5×10^{-7} M β-funaltrexamine. The calculated receptor constants indicated a "high-affinity, low intrinsic efficacy" profile (i.e. a potential partial agonist property) for endomorphin-1, an intermediate character for endomorpin-1-ol and full agonism for DAMGA and DAMGO. Apparently, a higher receptor fraction remained accessible for endomorphin-1 (42.8%) than for the -ol congener (14.0%), DAMGO (20.2%) and DAMGA (14.1%) after partial inactivation. © 2001 Published by Elsevier Science B.V.

Keywords: Endomorphin-1-ol; Endomorphin-2-ol; DAMGO ((D-Ala^2, MePhe^4, Gly^5-ol)-enkephalin); DAMGA ((D-Ala^2, MePhe^4, Gly^5-NH$_2$)-enkephalin); Ileum, guinea-pig; Vas deferens, mouse; β-Funaltrexamine; Receptor constant

1. Introduction

Endomorphins were the first mammalian brain opioid peptides with highly μ-opioid receptor selective agonist properties (Zadina et al., 1997). Endomorphin-1 and-2 displayed an activity pattern in isolated organs characteristic of a μ-opioid receptor agonist, produced supraspinal, μ-opioid receptor-mediated analgesia (Zadina et al., 1997; Rónai et al., 1998/99) and several other central nervous system actions as well (e.g. Champion et al., 1997). At first sight, their agonist behavior could be described as that of a full agonist; however, there were indications that they may act in some systems as partial agonists (Narita et al., 1998). Since Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO, Kosterlitz and Paterson, 1981) has long been known as a prototype μ-opioid receptor-selective synthetic peptide with properties closely matching the criteria for a full agonist, we synthetised the -ol derivatives of endomorphins and tested them in isolated organs. Further experiments were aimed at revealing potential partial agonist properties among the investigated peptides. To attain this, we used an indirect approach to determine agonist affinity (characterized in terms of K_A); by relating this receptor-related constant to agonist potency, we may assess the intrinsic efficacy of an agonist. To obtain receptor constants for the agonists (Furchgott and Bursztyn, 1967) in the mouse vas deferens, we used the strategy of partial, irreversible inactivation of μ-opioid receptors by β-funaltrexamine (Portoghese et al., 1980), which has been characterized mainly as an irreversible μ-opioid receptor antagonist and reversible κ-opioid receptor agonists (Takemori et al., 1981; Ward et al., 1982a,b; Hayes et al., 1985; Franklin

* Corresponding author. Tel.: +36-1-210-4416; fax: +36-1-210-4412.
E-mail address: RONAND@PHARMA.SOTE.HU (A.Z. Rónai).

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and Traynor, 1991). The -ol derivatives, similarly to the parent endomorphin peptides, were highly μ-opioid receptor-selective agonists in both isolated organs. They were nearly equipotent in mouse vas deferens and guinea-pig ileum, whereas endomorphins were significantly more potent in guinea-pig ileum than in mouse vas deferens (see also Rónai et al., 1998/99). After partial, irreversible inactivation of μ-opioid receptors in mouse vas deferens by 5×10^{-7} M β-funaltrexamine , the calculated receptor constants indicated a "high-affinity, low-intrinsic-efficacy" trend for endomorphin-1 (i.e. a potential partial agonist property), an intermediate character for endomorphin-1-ol and full agonism for DAMGA (Tyr-D-Ala-Gly-MePhe-Gly-NH_2) and DAMGO. A further distinctive property of endomorphin-1 was the higher accessible receptor fraction after partial receptor pool inactivation.

The novel brain peptide endomorphins seem to possess properties unlike those of hitherto known neuropeptides, both biologically and biochemically. The present analyses were aimed at revealing potentially distinctive pharmacological features. For this purpose, the determination of receptor constants proved to serve as a useful tool.

2. Materials and methods

2.1. Materials

Synthesis of Tyr-Pro-Trp-Phe-NH_2, Tyr-Pro-Phe-Phe-NH_2 and Tyr-D-Ala-Gly-MePhe-Gly-NH_2: Peptide amides were synthetised on Rink amide resin (Reanal, Hungary) using our standard Fmoc-protocol (Bódi et al., 1997). Synthesis of Tyr-Pro-Trp-Phe-ol (endomorphin-1-ol) and Tyr-Pro-Phe-Phe-ol (endomorphin-2-ol): Fmoc-Phe-ol was synthetised from Fmoc-Phe-OCH_3 following literature procedures (Soucek et al., 1990). Fmoc-Phe-ol was coupled to 2-chlorotrityl resin (Orosz and Kiss, 1998); elongation of the peptide chain was performed as described above. The resin–peptide bond was cleaved in dichloromethane containing 0.1% trifluoroacetic acid. The peptides were isolated by precipitation from the cleavage cocktail by diethyl ether. The crude peptides were purified by preparative HPLC (high-prformance liquid chromatography) on a C_{18} reverse-phase column using acetonitrile-water gradient containing 0.05% trifluoroacetic acid; their composition was verified by mass spectrometry and amino acid analysis.

Deltorphin-II was kindly supplied by G. Tóth of Biological Research Center of Hungarian Academy of Sciences, Szeged. Naltrexone hydrochloride was a gift from Du Pont Pharmaceuticals (Geneva, Switzerland). β-funaltrexamine hydrochloride was obtained from Tocris Cookson (Bristol, UK).

Protected amino acid derivatives Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Phe-OH, Fmoc-*N*-methyl-phenylalanine (Fmoc-MePhe-OH) were obtained from Reanal or synthetised in our laboratory. Fmoc-Trp(Boc)-OH was obtained from Novabiochem (United Kingdom). All the other substances used were of analytical grade and obtained either from the Sigma-Aldrich (St. Louis, USA) or Reanal.

2.2. Methods

2.2.1. Isolated organs

Mouse vas deferens. Vasa taken from CFLP (Carworth Europe Farm, Lanne-Patter, ICI Alderly Park I stock) mice weighing 35–40 g were prepared and used as described previously (Rónai et al., 1977). In brief, the organs (a single vas/bath.) were mounted under an initial tension of 0.1 g in Mg^{2+}-free Krebs' solution aerated with carbogen (O_2:CO_2 = 95:5) at 31°C. Field electrical stimulation (upper ring, lower straight wire electrode arrangement) was used. The parameters of stimulation were as follows: pairs (100 ms pulse distance) of rectangular impulses (1-ms pulse width, 9 V/cm, i.e. supramaximal intensity) were repeated by 10 s.

Longitudinal muscle strip/Auerbach plexus of guinea-pig ileum. Male, non-albino guinea-pigs weighing 400–500 g were used. The strips were prepared according to Paton and Vizi (1969) and the experimental conditions were the same as used previously (Rónai et al., 1977). In brief, 25–40 mm long strips were mounted under an initial tension of 0.8 g in Krebs' solution aerated with carbogen at 36°C. The parameters of field stimulation were as follows: supramaximal (1-ms pulse width, 9 V/cm intensity) rectangular impulses delivered at 0.1 Hz frequency. Throughout the experimentation, the ethical guidelines based on the Helsinki declaration were observed; the local ethical board approved the protocols.

2.2.2. Experimental paradigms

In the isolated organ series, 30–40 min equilibration was used for mouse vas deferens, 45–60 min for guinea-pig ileum under stimulation. The dose–response curves for the agonists were constructed in noncumulative manner; the drug exposure was less than 2 min, the administration cycle 12–18 min, with three to four interim washes. The preparations were equilibrated with naltrexone for 20 min; the single-dose method (Kosterlitz and Watt, 1968) was used for assessing the antagonism in guinea-pig ileum, whereas complete dose–response curves were taken with the agonists also in the presence of antagonist in mouse vas deferens. Based on the results of pilot experiments, 30-min exposure to 5×10^{-7} M β-funaltrexamine was chosen. In the control period, always in paired arrangement, endomorphin-1, endomorphin-1-ol, DAMGA and DAMGO dose–response curves were constructed from four to five preset concentrations, followed by a single concentration of deltorphin-II. In one paired experiment, a crossover design was used, i.e. the same preparation received alternately endomorphin and the -ol derivative;

Table 1
The opioid characteristics of endomorphin derivatives, (D-Ala^2, MePhe^4, Gly^5-ol)-enkephalin and its Gly^5-NH_2 congener in longitudinal muscle strip of guinea-pig ileum (GPI) and mouse vas deferens (MVD)

Peptide	GPI		MVD		GPI/MVD[a] potency ratio
	IC_{50} (nM)[b]	Ntx K_e (nM)[c]	IC_{50} (nM)[b]	Ntx K_e (nM)[c]	
Endomorphin-1	14.7 (9.4–23.2, $n=9$)	0.20 (0.17–0.23, $n=4$)[d]	29.4 (17.9–48.3, $n=16$)	0.21 (0.19–0.24, $n=4$)[d]	2.0
Endomorphin-1-ol	61.2 (45.1–83.0, $n=8$)	0.31 (0.25–0.37, $n=8$)	80.6 (57.1–113.6, $n=16$)	0.42 (0.33–0.53, $n=6$)	1.3
Endomorphin-2	11.0 (7.3–16.7, $n=6$)	0.41 (0.27–0.61, $n=4$)[d]	21.9 (15.5–30.9, $n=8$)	0.27 (0.24–0.29, $n=4$)[d]	2.0
Endomorphin-2-ol	48.2 (25.8–90.1, $n=8$)	0.40 (0.27–0.60, $n=7$)	49.6 (31.3–78.7, $n=6$)	0.29 (0.20–0.42, $n=6$)	1.0
DAMGA[e]	7.99 (4.95–12.9, $n=6$)	0.31 (0.25–0.39, $n=4$)	18.6 (14.1–24.7, $n=11$)	0.40 (0.26–0.59, $n=6$)	2.3
DAMGO[e]	29.2 (17.5–48.6, $n=12$)	0.30 (0.27–0.33; $n=5$)	59.8 (41.6–85.9, $n=8$)	0.33 (0.29–0.37; $n=6$)	2.1

[a] The IC_{50} in mouse vas deferens divided by the one in guinea-pig ileum.
[b] 50% inhibitory concentration; geometric means and 95% confidence intervals are listed.
[c] Equilibrium dissociation constant; geometric means and 95% confidence intervals.
[d] Taken, with permission, from Rónai et al. (1998/99).
[e] DAMGA: (D-Ala^2, MePhe^4, Gly^5-NH_2)-enkephalin; DAMGO: the Gly^5-ol-derivative.

these results were not pooled with the others. Exposure to β-funaltrexamine was followed by a 40–60 min washout period with 8–12 washes. A preset criterion for inclusion, which has been recommended by Ward et al. (1982a), was an at least 80% recovery. After recovery, a selected dose (5×10^{7} M of endomorphin-1, 2×10^{-6} M of -ol derivative, 2.5×10^{-7} M of DAMGA and 5×10^{-7} M of DAMGO) of agonists were repeated until the responses became stabilized; it took two to four repetitions. Thereafter, the agonist effects were tested at three further, preset concentration levels, and the administration cycle was terminated by deltorphin-II.

2.2.3. Evaluation

In the isolated organs, the IC_{50} values were calculated from the logarithmic regressions of individual dose–response curves. For determining the parameters of antagonism (dose ratio, DR and equilibrium dissociation constant, K_e) by the competitive opioid receptor antagonist naltrexone, either the single-dose method (in guinea-pig ileum, Kosterlitz and Watt, 1968) was used or they were calculated from complete dose–response curves taken in the absence and presence of antagonist (in mouse vas deferens, Arunlakshana and Schild, 1959).

For the pooled IC_{50} and K_e values, the geometric means and the 95% confidence intervals (Fleming et al., 1972) were calculated. In order to assess the receptor constants from the dose–response relationships obtained before and after β-funaltrexamine exposure, a sigma plot (ver. Gompertz 3) program run under Windows '95 was used for curve fitting to the respective set of points. For the "double reciprocal" plot ($1/A$ vs. $1/A'$, Furchgott and Bursztyn, 1967; Tallarida and Jacob, 1979; Tallarida, 1982), the equieffective concentrations of agonists before (A) and after (A′) β-funaltrexamine were obtained by producing intercepts at the suitable segment of dose–response curves in 5% or 10% ordinate increments. From the linear regression of double reciprocal plot, the active receptor fraction after β-funaltrexamine (q) is given as $1/\text{slope}$; for convenience, it was expressed in percent. The apparent dissociation constant of agonist (K_A) is given by $(\text{slope}-1)/y$ intercept.

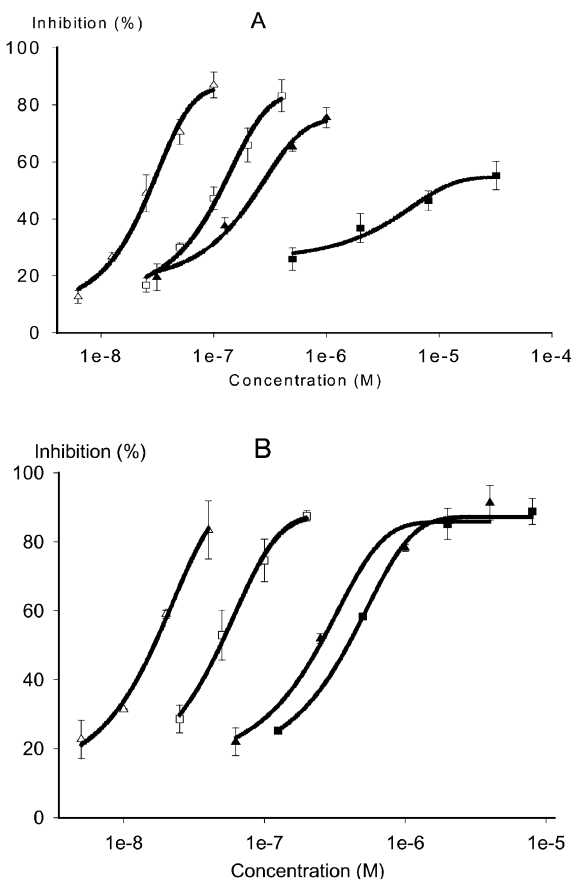

Fig. 1. The inhibitory dose–response curves of endomorphin-1 (△,▲), endomorphin-1-ol (□,■) (panel A), DAMGA (△,▲) and DAMGO (□,■) (panel B) in the mouse vas deferens before (open symbols) and after (closed symbols) 30-min incubation with 5×10^{-7} M β-funaltrexamine. Sigma plot was used for curve fitting. Points represent the mean, vertical lines the S.E.M. of data obtained in three to four independent experiments.

For statistical comparisons, either paired *t*-test or analysis of variance (ANOVA) followed by Newman–Keuls post hoc test was used.

3. Results

3.1. General in vitro opioid pharmacology

The -ol derivatives were slightly less potent agonists than the parent (amidated) endomorphins in both bioassays. The -ol derivatives were nearly equipotent agonists in the two preparations, endomorphins were slightly more potent in guinea-pig ileum than in mouse vas deferens (see also Rónai et al., 1998/99). Of DAMGA/DAMGO pair, also, the amide was the more potent one. The slopes of logarithmic regressions of endomorphin dose–response curves tended to be higher in guinea-pig ileum than those of corresponding -ol-peptides; however, this tendency attained statistical significance only for the endomorphin-1/ol pair (33.8 [29.8–38.3], $n = 4$ vs. 24.1 [19.7–29.5], $n = 8$, geometric means and 95% confidence intervals). In mouse vas deferens no such a tendency was found. The K_e values of opioid antagonist naltrexone against the peptides fell into the range of 0.2–0.4 nM in both preparations (Table 1), indicating the principal involvement of μ-opioid receptor type (see also Discussion).

3.2. Determination of receptor constants

If the receptor-type selection is the same for two or more agonists, one of the possible explanations for the higher relative potency in guinea-pig ileum (where the pool of "spare" μ-opioid receptors is higher than in mouse vas deferens, Miller et al., 1986) is a potential partial agonist property. To follow up this possibility, we used the strategy of partial irreversible receptor inactivation by β-funaltrexamine. Mouse vas deferens was the preparation of choice partly because of the lower pool of spare μ-opioid receptors and partly because of relative sparseness of κ-opioid receptors. The first property was expected to result in a proportionately higher "net" inactivation of functional μ-opioid receptors at a given β-funaltrexamine concentration, the latter to facilitate the washout after the reversible κ-opioid receptor agonist action by β-funaltrexamine The chosen concentration of β-funaltrexamine i.e. 5×10^{-7} M caused $32.2 \pm 1.4\%$ ($n = 11$, arithmetic mean ± S.E.M.) inhibition, with a moderate tendency of recovery throughout the 30-min exposure ($26.8 \pm 2.2\%$ inhibition at the endpoint). After 40–60 min and 8–12 washes, there was a recovery to $94.9 \pm 1.9\%$ of control; no exclusion was necessary since recovery was higher than 80% in each experiment. The dose–response curves of endomorphin-1/-ol pair before and after β-funaltrexamine exposure are shown in Fig. 1, panel A, whereas those for

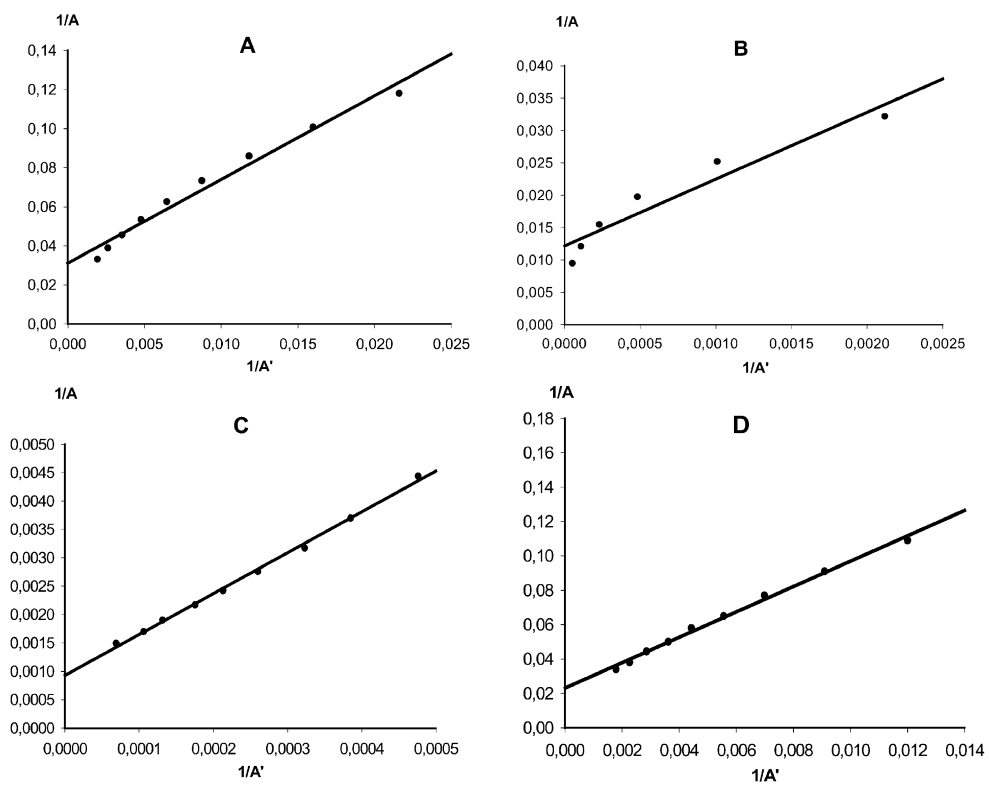

Fig. 2. Double reciprocal plot of equieffective concentrations of endomorphin-1 (panel A), endomorphin-1-ol (panel B), DAMGA (panel C) and DAMGO (panel D) obtained before (A) and after (A′) incubation of mouse vasa deferentia with 5×10^{-7} M β-funaltrexamine. The presented data were obtained is single, typical experiments.

Table 2
Receptor constants for endomorphin-1, endomorphin-1-ol, (D-Ala^2, MePhe^4, Gly^5-NH$_2$)-enkephalin and its Gly^5-ol congener in mouse vas deferens

Peptide	Regression of 1/A vs. 1/A′ plot[a]	K_A (nM)[b]	q[c] (Active receptor fraction, %)	K_A/IC$^d_{50}$
Endomorphin-1	$y = 2.46 \pm 0.42\ x + 0.030 \pm 0.0041$ ($n = 4$) ($r^2 = 0.85/7, 0.95/5, 0.95/6, 0.96/6$)	44.1 (22.1–88.0)	42.8 (31.3–58.6)	1.71 (1.06–2.74)
Endomorphin-1-oll	$y = 7.50 \pm 1.08\ x + 0.0097 \pm 0.00049$ ($n = 4$) ($r^2 = 0.87/6, 0.91/7, 0.93/7, 0.97/6$)	632 (465–859)	14.0 (10.3–18.9)	5.58 (4.66–6.68)
DAMGA	$y = 7.26 \pm 0.85\ x + 0.018 \pm 0.003$ ($n = 4$) ($r^2 = 0.99/7, 0.99/9, 0.99/8, 0.95/8$)	355 (274–461)	14.1 (11.3–17.6)	19.2 (14.6–25.2)
DAMGO	$y = 5.13 \pm 0.83\ x + 0.0072 \pm 0.0015$ ($n = 3$) ($r^2 = 0.98/10, 0.99/6, 0.99/9$)	589 (423–821)	20.2 (15.6–26.3)	12.3 (8.7–17.4)

[a] Plots were constructed from the reciprocals of equieffective concentrations of agonist before (A) and after (A′) 30-min incubation with 5×10^{-7} M β-funaltrexamine. The equation is given as the arithmetic mean ± S.E.M of slope and y intercept parameters obtained in individual experiments. The individual r^2 parameters and the number of points taken for a given individual construct appear in parenthesis.

[b] Dissociation constant of agonist, calculated as (slope − 1)/intercept on the ordinate. Geometric means and 95% confidence intervals are listed.

[c] Fraction of receptors available for the agonist after β-funaltrexamine incubation, calculated as 1/slope. Geometric means and 95% confidence intervals.

[d] For the calculation the IC$_{50}$, obtained in the same preparation, was used.

the DAMGA/DAMGO pair in panel B. The dose–response curves were shifted to the right; there was a slight reduction of slope and maximum effect in the case of endomorphin-1, whereas these parameters were markedly changed in the case of endomorphin-1-ol. The reduction in the effectiveness of δ-opioid receptor agonist deltorphin-II was moderate though statistically significant (88.3 ± 1.5% inhibition before vs. 67.7 ± 2.4% after β-funaltrexamine exposure at 10^{-9} M concentration of agonist; $p < 0.05$, $n = 16$, paired t-test). To obtain receptor constants for the two agonists, according to Furchgott and Bursztyn (1967), first equiactive concentrations of respective agonists before (A) and after (A′) β-funaltrexamine incubation were determined in individual, paired experiments (for details, see Methods). Typical "double reciprocal" plots (1/A vs. 1/A′) for endomorphin-1 (panel A), endomorphin-1-ol (panel B), DAMGA (panel C) and DAMGO (panel D) are shown in Fig. 2. The pooled parameters of individual linear regressions and the calculated receptor constants are given in Table 2.

By the calculated parameters, endomorphin-1 has approximately 14 times higher affinity to the μ-opioid receptors in mouse vas deferens than the parent -ol derivative. There was no such a difference between the affinities of DAMGA/DAMGO pair; the K_A values for both peptides fell into the same range as the one for endomorphin-1-ol. Furthermore, apparently, a much higher percentage of μ-opioid receptors remain available for endomorphin-1 after β-funaltrexamine incubation than for endomorphin-1-ol, DAMGA or DAMGO ($p < 0.05$ by ANOVA followed by Newman–Keuls test). The same difference was also found in the "crossover" type of experiment (not shown, see Methods) underlying that the difference is not due to individual variations across preparations. The ratio relating agonist potency (IC$_{50}$) to agonist affinity (K_A) reflects a "high affinity, low efficacy" profile for endomorphin-1 ($p < 0.005$ vs. DAMGA, $p < 0.05$ vs. DAMGO), an intermediate profile for endomorphin-1-ol ($p < 0.01$ vs. DAMGA, $p < 0.05$ vs. DAMGO), whereas a "low affinity, high efficacy" one for DAMGA and DAMGO, DAMGA being the "fuller" agonist of the two ($p < 0.05$).

4. Discussion

In the present series of experiments the -ol-derivatives of endomorphins, similarly to the parent natural peptides were μ-opioid receptor-selective agonists both in the mouse vas deferens and guinea-pig ileum bioassays. However, there was no improvement, even a slight reduction in agonist potencies as a consequence of amide -ol modification. It should be kept in mind that endomorphins are "ab ovo" C-terminally amidated, which is a structural feature promoting μ-opioid receptor-type selection (Rónai et al., 1979).The very same tendency is apparent also in the case of (D-Ala^2, MePhe^4, Gly^5-ol)-enkephalin/(D-Ala^2, MePhe^4, Gly^5-NH$_2$) pair; the amide is consistently more potent in both isolated organs.

Since there were indications in the literature (Narita et al., 1998) that endomorphins may possess partial agonist properties, we decided to analyse this possibility because the full or partial agonist character of an agonist is an important issue both from theoretical and practical point of view. For agonists where the antagonist property is manifested below or within the concentration range where the agonist effect is exerted, the distinction is easily made by simple pharmacological means; this, certainly, does not apply for endomorphins. The improving methodology both in biochemical pharmacology (first the "sodium shift" of receptor binding then the (^{35}S) GTP binding) and traditional pharmacology (partial receptor pool inactivation) gave useful tools to differentiate in more complex cases. Full or partial agonism stem from the drug-receptor-related factor commonly known as intrinsic efficacy (Stephenson,

1956; Furchgott and Bursztyn, 1967; Tallarida and Jacob, 1979; Tallarida, 1982; Kenakin, 1999). In the present series of experiments, we used the partial, irreversible receptor pool inactivation strategy (Furchgott and Bursztyn, 1967). When using an irreversibly acting agent as a tool for determining receptor constants of agonists in isolated tissues the following criteria should be met: (1) The agonist should act at a single, homogenous receptor population; (2) The irreversible agent should inactivate a fraction of receptors without altering the affinity of agonist to the remaining receptor population or interfering with the molecular events involved in signal transduction; and (3) The functional chain mediating the biological response distal to the receptor should be left intact. β-funaltrexamine and chlornaltrexamine have been synthetised (Portoghese et al., 1979, 1980) with the aim of producing irreversible opioid receptor antagonists. Interaction with δ-opioid receptors is present and occurs regularly in the same concentration range where irreversible μ-opioid receptor blockade is produced (Hayes et al., 1985). Corbett et al. (1985) have found that while μ-opioid receptor agonists lost potency irreversibly in guinea-pig ileum after β-funaltrexamine incubation, there was, apparently, no loss in ligand binding sites in tissue homogenate. β-funaltrexamine action has, therefore, been attributed to an interference with the receptor-effector coupling system in this tissue rather than a blockade of binding sites. However, as it was shown by a carefully conducted reinvestigation (Franklin and Traynor, 1991), this anomaly was due to the incubation conditions; in fact, the presence of Na^+ and Gpp(NH)p β-funaltrexamine did reduce binding capacity also in tissue homogenate. Under our experimental conditions, β-funaltrexamine exerted a slighter reversible inhibitory effect on its own in the mouse vas deferens bioassay, when tested in the concentration range of 2×10^{-7}–2×10^{-6} M, than it was expected from the previous reports on the actions of drug in the same isolated organ. Furthermore, the irreversible impairment of δ-opioid receptor function was also unexpectedly moderate; there was only a slight reduction in the effectiveness of deltorphin-II tested in a concentration approximately twice the IC_{50} value. Thus, partly because of these factors, partly because of the reportedly lower μ-opioid receptor pool in the mouse vas deferens, we decided to determine the β-funaltrexamine endomorphin-1/-1-ol, DAMGA/DAMGO interaction in this isolated organ. From the receptor constants calculated from the double-reciprocal plots of equiactive agonist concentrations before and after β-funaltrexamine incubation, it became evident that endomorphin-1 differed from endomorphin-1-ol and the DAMGA/DAMGO pair not just in one, but two, receptor-related parameters. While the K_A values of endomorphin-1-ol, DAMGA and DAMGO fell into 300–600 nM range, the affinity of endomorphin-1 was approximately by one order of magnitude higher. Furthermore, the receptor fraction available after β-funaltrexamine treatment was higher for endomorphin-1 (42.8%) than for the other tested agonists (14.0–20.2%). The latter finding is rather difficult to interpret even speculatively. The primary structures of different opioid receptor types are known and the alignment of different receptor protein domains relative to the cell membrane can be predicted with good probability (for review, see Reisine and Bell, 1993; Uhl et al., 1994; Quock et al., 1999). Ligand-binding selectivity of receptor types stems mainly from binding pockets of well-defined geometry and their occlusion by strategically located amino acids or short segments rather than from gross amino acid composition differences (Metzger and Ferguson, 1995; Quock et al., 1999). It is also known that β-funaltrexamine interacts irreversibly with a single amino acid residue, Lys 233 in the rat μ-opioid receptor protein (Chen et al., 1996). Lys 233 is located at the extracellular end of the fifth transmembrane domain, in the close proximity of "morphinan binding pocket"; it is not involved directly in ligand binding. The domain responsible for the high-affinity binding of a "non-morphinan" ligand, DAMGO resides within the first extracellular loop of μ-opioid receptor (Onogi et al., 1995); the obstruction of this region by Lys 108 in cloned rat δ-opioid receptor is responsible for the binding selectivity profile of this receptor type (Minami et al., 1996). Irreversible interaction of β-funaltrexamine with Lys 233 renders both the "morphinan binding pocket" and the "non-morphinan -binding domain(s)" of μ-opioid receptor fractionally inaccessible to ligands. Assuming that endomorphin-1 and endomorphin-1-ol are assigned to the same binding pocket, one of the possible explanations for the different fractional receptor accessibilities is that possibly endomorphin-1 has a more folded, geometrically more compact structure. Finally, by relating the agonist affinities (K_A values) to agonist potencies (IC_{50} values), a high intrinsic efficacy (full agonism) can be predicted for DAMGA and DAMGO, an intermediate character for endomorphin-1-ol, whereas a potential partial agonism for endomorphin-1.

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