Bioorganic & Medicinal Chemistry Letters 27 (2017) 2119–2123

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Original endomorphin-1 analogues exhibit good analgesic effects with minimal implications for human sperm motility

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ARTICLE INFO

Article history:
Received 19 February 2017
Revised 17 March 2017
Accepted 23 March 2017
Available online 27 March 2017

Keywords:
Endomorphin-1 analogues
Analgesic
Sperm motility

ABSTRACT

To search a novel analgesic characterizes the effects on human sperm motility as minimal as possible. A new class of endomorphin-1 (EM-1) analogues was synthesized by combining successful chemical modifications including N-terminal guanidino modification, Phe^4 was chlorinated, replaced of L-Pro^2-Trp^3 by D-Ala^2-Gly^3 or D-Pro^2-Gly^3 at position 2 and 3. Their bioactivities were measured by radioligand binding assay, metabolic stability, antinociception activity and sperm motility effects. In radioligand binding assays, analogue GAGP shown a μ-opioid receptor affinity about 17.7-fold higher and a 57.3-fold higher δ-opioid receptor affinity than EM-1. In the metabolic stability assays, GAGP had the longest half-lives and 16.6-fold higher than EM-1. In the tail-flick test in mice, GAGP showed the best analgesia. In sperm motility assays, the group of GAGP (10^{-5}, 10^{-7} mol/L) decreased of the percentage of a + b grade, and no significant when compared with initial value. In GAGP (10^{-6} mol/L) group, sperm motility was progressively increased, although it was not statistically significant. But at the groups of morphine (10^{-7} mol/L) and GAGD (10^{-7} mol/L), these caused significant reduction between 0 and 90 min. We found that analogues GAGP, activating μ-opioid receptor and partial δ-opioid receptor, exhibit good analgesic effects with minimal implications for human sperm motility. It might be important in potential application as drug candidates of analgesic without implications for human sperm motility.

More than a third of infertile couples are due to male factors. Male infertility mainly include genetic diseases, anatomical defects, injuries, testicular sperm as well as hormonal dysfunction.[1] Sperm motility is widely used to predict male factor infertility, because it is essential for natural reproduction.[2,3] Drug addicts who exposure to opiates show the asthenozoospermia (reduced sperm motility).[4] The most people know physiological effects of Endogenous opioid peptides (EOPs) are analgesia, endogenous opioid peptides are present in various tissues and organs of the male and female reproductive tract, suggesting that they may regulate some of the processes involved in reproductive function.[5]In fact, the opioid system can participate in the regulation of reproductive physiology at multiple levels, for example, at the levels of the central nervous system, at the testes level and at sperm level, operating as a multi-messenger system. In previous studies, the three principal types of opioid receptors (MOR, DOR and KOR) have been found in the membranes of human sperm cells.[6,7] Some studies reported that high concentrations of β-endorphin and enkephalin inhibited sperm motility.[8] In the clinic, μ-opioid-receptor analgesics, for example, morphine, fentanyl, alfentanil, or sufentanil are the most common therapeutic analgesic for acute pain.[9,10] However, morphine fentanyl, alfentanil, or sufentanil inhibited sperm motility, compared to the control group.[6,11] Interestingly, a low dose of δ-agonist met-enkephalin was found to be sufficient to maintain sperm motility.[12]

The MOR endogenous tetrapeptides, endomorphin-1 (EM-1, H-Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2, H-Tyr-Pro-Phe-Phe-NH_2), were isolated from the bovine brain and the human cortex in 1997.[13,14] Endomorphins (EMs), containing EM-1 and EM-2, displayed profound antinociceptive effects in neuropathic pain and inflammatory pain.[10,15–18] But EM-1 is still with limitation of poor metabolic stability and relative inability to cross the blood-brain barrier (BBB) into the central nervous system (CNS) as analgesics.

The aim of this study was to develop an analgesic with minimal implications for human sperm motility, a novel series of endomorphins analogues have been synthesized by combining successful chemical modifications,[19–22] N-terminal guanidino modification,[23] Phe^4 was chlorinated, replaced of L-Pro^2-Trp^3 or L-Pro^2-Phe^3 by

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http://dx.doi.org/10.1016/j.bmcl.2017.03.067
0960-894X/

Table 1
Amino acid sequences and TOF MS of all analogues.

Pesptides	Sequences	TOF MS $[M+H]^+$	
		Calcd	Found
EM-1	Tyr-Pro-Trp-Phe-NH_2	611	611.1
GAGP	N^{α}-Amidino-Tyr-D-Ala-Gly-Phe(4-Cl)-NH_2	531	532.4
GAGD	N^{α}-Amidino-Tyr-D-Ala-Gly-Phe-NH_2	497	498.4
HAGD	H-Tyr-D-Ala-Gly-Phe-NH_2	456	456.3
HAGP	H-Tyr-D-Ala-Gly-Phe(4-Cl)-NH_2	489	490.1
GDGD	N^{α}-Amidino-Tyr-D-Pro-Gly-Phe-NH_2	523	524.1

D-Ala^2-Gly^3 or D-Pro^2-Gly^3 at position 2 and 3, in attempts to develop a long-term analgesia under subcutaneous (s.c.) and intracerebroventricular (i.c.v) administration. In addition, in these EM-1 analogues, we also evaluated the effect of partial agonist (activating μ-opioid and δ-opioid) on human sperm motility to find the analgesics without the toxicity.

Amino acid sequences (shown in Table 1) of EM-1 and all its analogues were obtained by solution-phase methods with segment-coupling peptide synthesis strategy in School of Basic Medical Sciences, Lanzhou University (Gansu, China). Wistar rats and Male Kunming mice were obtained from Animal Center of Medical College of Lanzhou University in China. Following the literature method, membranes were prepared from Wistar rat brain except cerebellum.[24] The experiments processes are in Supplement materials.

EM-1 and all its analogues binding affinities for μ- and δ-opioid receptors are shown in Table 2. Analogue HAGD shown a Ki (μ) being about 2.9-fold lower and a Ki(δ) being 1.5-fold lower compared to the EM-1. Analogue GAGP shown a μ-opioid receptor affinity about 17.7-fold higher and a 57.3-fold higher δ-opioid receptor affinity than EM-1. The EM-1 analogue HAGP with a Ki (μ) being about 5.0-fold lower compared to the EM-1 parent peptide, and Ki(δ) higher than 1000 nM. GAGD displayed 14.7-fold higher μ-opioid receptor affinity and 6.9-fold higher δ-opioid receptor affinity than EM-1. GDGD showed a Ki (μ) being about 39.9-fold higher and a Ki(δ) being 1.3-fold lower compared to the EM-1.

Table 2 summarizes the half-lives determined for all of the test peptides in 15% mouse brain homogenate and 100% mouse serum. Within the brain homogenate, EM-1 disappeared rapidly, with half-lives being 23.54 min. All analogues showed significant increase in half-lives than EM-1. HAGD had the half-lives which were 259.73 min. GAGP had the longest half-lives which were 390.91 min and 16.6-fold higher than that of EM-1. HAGP showed 1.2-fold lower half-lives than GAGP. GAGD and GDGD had a half-lives about 364.26 min and 296.11 min, respectively. In the plasma, EM-1 was also degraded rapidly, with half-lives being only 6.09 min. HAGD had the longest half-lives which was 262.61 min and 43-fold higher than that of EM-1. GAGP showed 234.63 min in half-lives. HAGP and GAGD had the half-lives which were 239.23 min and 232.37 min. GDGD showed the shortest half-lives which were about 220.94 min in all analogues.

Antinociceptive activities of EM-1 and its analogues were tested in the tail-flick test. Time course of the antinociceptive effect in s.c. or i.c.v injection, and area under the curve (A.U.C.) of EM-1 and its analogues were shown in Fig. 1 and Fig. 2 respectively.

Administered an i.c.v. injection of 0.5, 1.25, 2.5, 10 nmol/kg of GAGD and GAGP, the results show that the analogues of GAGD and GAGP had a long history of super analgesia in dose of 10 nmol/kg, the biggest analgesic effect from the beginning 10 min of the administration until 80 min after the administration, that has been maintained at between 90% and 100% (Fig. 1 a and b). Then, further examined the analgesic activity of GAGD and GAGP at low concentrations compared with EM-1. It can be seen in Fig. 1 d and e, the analgesic activity of analogues GAGD and GAGP under dose of 2.5 nmol/kg is better than EM-1 under does of 10 nmol/kg, and appear long duration of action. The analgesic effect of analogue HAGD, HAGP at a concentration of 10 nmol/kg was weak relative to other EM-1 analogues (Fig. 1.c).

The analgesic activity was measured by injection drugs under s.c. Firstly, the best analgesic effect of GAGP, GAGD after i.c.v administration, was chosen continue to study the analgesic effect of s.c. (Fig. 2 a and b). The results showed the analgesic effect was the best and the duration was the longest when the dosage was 10 mg/kg. From the results of pre experiment, morphine can produce obvious analgesic effect in 5 mg/kg. Therefore, we chose 5 mg/kg to compare the analgesic activity of morphine and EM-1 analogues. Results displayed that the analgesic effect of analogues HAGD and HAGP was inferior to morphine (Fig. 2.c). However, the analogues GAGP, GAGD showed a very good analgesic effect, not only analgesic action stronger than morphine, but also the duration longer than morphine (Fig. 2.d). As can be seen from (Fig. 2.e), the strongest analgesic effect was GAGP under the same dose.

The result of motility effects (A plus B grades) of the GAGD, GAGP and the morphine shown in Table 3. The sperm motility decreased over time according to data of Table 3, no statistically differences ($P > 0.05$) was found from 0 min to 90 min in control, morphine (10^{-5} mol/L), morphine (10^{-6} mol/L), GAGD (10^{-5} mol/L), GAGD (10^{-6} mol/L), GAGP (10^{-5} mol/L) and GAGD (10^{-7} mol/L) groups, but the decrease of the sperm motility on group of addition of GAGD (10^{-7} mol/L) and morphine (10^{-7} mol/L) after 90 min have statistically significant ($P < 0.05$) compare with initial value. Of particular interest here, in the GAGP (10^{-6} mol/L) group, the results of sperm motility were exact opposite of other groups, it was progressively increased, although it was not statistically significant.

The EM-1 displayed profound antinociceptive effects in neuropathic pain and inflammatory pain via μ-opioid receptors.[25,26] Antinociceptive effects of analogues were depended on the number of activated μ-opioid receptor and metabolic stability in the plasma and so on. EM-1 expressed short acting antinociceptive activities, due to low metabolic stability. In the present study, it

Table 2
EM-1 and all its analogues binding affinities for μ- and δ-opioid receptors.

Peptides	Ki(μ)(nM)	Ki(δ)(nM)	Ki(δ)/Ki(μ)	Half-livf (min)	
				15% brain homogenate	Plasma
EM-1	6.19 ± 0.36	5775 ± 660	933	23.54 ± 0.25	6.09 ± 0.31
GAGP	0.35 ± 0.12	100.8 ± 16	288	390.91 ± 88.75	234.63 ± 46.08
GAGD	0.42 ± 0.13	840 ± 65	2000	364.26 ± 94.32	232.37 ± 6.54
HAGD	2.11 ± 0.17	3800 ± 320	1801	259.73 ± 41.01	262.61 ± 25.20
HAGP	1.24 ± 0.27	(−)[R1]	(−)[R1]	317.61 ± 41.17	239.23 ± 20.84
GDGD	247 ± 26.64	4550 ± 293	18.41	296.11 ± 14.31	220.94 ± 30.09

μ-Opioid and δ-opioid receptor affinity and selectivity, half-lives in the plasma of EM-1 and all its analogues in vitro.
[R1] Ki(δ) higher than 1000 nM. The results are expressed as mean ± SEM for 8–12 measurements.