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Original

Uptake of radiolabelled endomorphins by experimental mammary adenocarcinoma

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Abstract

BACKGROUND: The aim of this study was to examine the accumulation of endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) labelled with radioiodine in tumour-bearing C3H/Bi mice.

MATERIAL AND METHODS: Mice C3H/Bi bearing transplantable mammary adenocarcinoma were used as animal models to study the interaction between μ-opioid receptors and endomorphin-1 and 2. The expression of the μ-opioid receptor in the tumours was confirmed by cross-linking assay and by RT-PCR technique.

RESULTS: The endomorphins showed relatively high tumour accumulation — about 5.2% of dose/g tissue for endomorphin-1 and about 3.8% for endomorphin-2. The ratio of tumour to muscle for endomorphin-2 reached the highest value (12.7) six hours after injection. For endomorhin-1 this ratio was the highest (7.5) three hours after injection. The cross-linking assay of [^{125}I]-labelled peptides with membranes, isolated from the mouse adenocarcinoma, followed by electrophoresis and autoradiography revealed the presence of a radioactive complex with molecular weight of about 65 kDa. This complex was detectable by polyclonal antibodies raised against the N-terminal end of a μ-opioid receptor. The expression of gene encoding μ-opioid receptor on mouse mammary adenocarcinoma was further confirmed by RT-PCR technique. The binding studies with membranes of mouse mammary adenocarcinoma cells have shown significantly higher B_{max} values for endomorphin-1 and endomorphin-2 (806 and 671, respectively) than for morphiceptin (131), a well-known specific μ-opioid receptor ligand.

CONCLUSIONS: Endomorphin-1 and 2 have shown a high affinity to the μ-opioid receptor present in mouse mammary adenocarcinoma. However, endomorphin-2 showed more promising characteristics in biodistribution studies.

Key words: μ-opioid receptor, endomorphin-1, endomorphin-2, mouse mammary adenocarcinoma

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Introduction

Opioid receptors were demonstrated on different tumour cells. Using specific radioligands, it was shown that MCF-7 breast cancer cells and histologically diverse lung cancer cells express multiple μ-, δ- and κ- types of these receptors [1–3]. The presence of high-affinity binding sites for morphine, endomorphin-1, and β-endorphin was disclosed in rat C6 glioma cells [4]. Human neuroblastoma cell line has highly stereospecific opiate binding sites [5].

It was also proved that some opioid agonists (ethylketocyclazocine, etorphine, modified enkephalins and morphine) may inhibit proliferation of human prostatic [6], lung [1] and breast cancer cell lines [3, 7]. This effect was antagonized by diprenorphine [6] and naloxone [3]. The above mentioned data suggest that opioids and their receptors may be involved in the growth and development of different types of cancers. This phenomenon opens the possibility to use the opioid receptor selective radioligands for tumour imaging. Recently, we have shown on mRNA and protein levels that μ-opioid receptor is expressed in mouse mammary adenocarcinoma [8]. Endomorphins, originally isolated from bovine and human brain cortex, are endogenous agonists of μ-opioid receptors [9, 10]. It has been reported that some peptides are connected with physiological modulation of pain, feeding responses, oxygen consumption and blood pressure regulation [9, 11–13]. Morphiceptin, another exogenous tetrapeptide

generated in the digestion tract from β-casomorphin, possesses morphine-like physiological activity with high affinity and selectivity to the μ-opioid receptor [14]. In the present study, the expression of m-opioid receptor on mRNA and protein levels has been checked in mouse mammary adenocarcinoma. In the same experiments, the binding of endomorphins by cancer tissue was determined in *in vivo* and *in vitro* studies.

Material and methods

Peptides

Endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) were obtained from the Calbiochem Corporation (La Jolla, California, USA)

Radiolabelling of peptides

Labelling of peptides with [^{125}I] or [^{131}I] was carried out by the chloramine T method [15] as previously described [8]. The labelled peptides were purified on a reverse-phase column and filtered through a 0.22 μm filter.

Animals

All experiments on living animals were carried out according to protocols approved by the local ethical committee. In the experiments, female C3H/Bi mice with transplantable mammary tumours were used. The animals were obtained from the Institute of Oncology, Warsaw, Poland and kept under standard conditions.

Tumour implantation

The tumours were homogenized in cold and sterile 0.9% NaCl (1:9 w/v). Tumour cell suspension was filtered through aseptic gauze and then injected subcutaneously (150 μl) into three different areas of the dorsal region of healthy mice, lightly anaesthetized.

Preparation of membranes

Tumour tissue was homogenized in 0.01 M potassium phosphate buffer (pH 7.4) with 5 mM $MgCl_2$ (1:9 w/v) and then centrifuged for 5 min at 5000 $\times$ g (4°C). The supernatant was then centrifuged for 40 min at 32.000 $\times$ g (4°C). The resulting pellet (crude membranes) was resuspended in the above mentioned buffer and stored at –80°C in 200 μl portions for further studies. Protein content was determined by Lowry's method using bovine serum albumin as a standard.

Receptor binding assay

Membranes (100 μg of protein) in 100 μl of 0.01 M potassium phosphate buffer (pH 7.4) with 5 mM $MgCl_2$ were incubated in triplicate for 2 h at room temperature with various concentrations of [^{125}I]-labelled ligand, with or without excess of nonradioactive ligand. Bound radioactivity was determined after centrifugation of membranes and washing with potassium phosphate buffer (2×). Finally, the membrane bound radioactivity was counted in a Wallac gamma counter.

Cross-linking assays of peptides with the receptor

50 μl of membranes (1 μg of proteins/μl) were incubated at room temperature for 90 min with 10 μl of the [^{125}I]-peptide (about 100,000 cpm). Next, the cross-linking agent ethylene glycol bis (succinimidyl succinate) was added up to 10^{-4} M and the samples were incubated at 4°C for 15 min [16]. The cross-linking step was stopped by addition of the sample buffer (0.0625 M Tris-HCl, pH 6.8; 4% SDS; 50% glycerol; 20% β-mercaptoethanol, and 0.25% bromophenol blue). The radioactive complex formation was detected by electrophoresis in 12.5% SDS-PAG followed by electroblotting onto polyvinylidene difluoride (PVDF) membranes (PerkinElmer Life Sciences) and autoradiography. Opioid receptor identification was done with the rabbit polyclonal anti-μ-opioid receptor antibody (Biosource, International) by Western blot technique [8].

RNA extraction

RNA was isolated from mammary adenocarcinoma tissue by Total RNA Prep Plus Minicolumn Kit (A&A Biotechnology, Poland).

RT-PCR

cDNA was synthesized by RevertAid™ cDNA Synthesis Kit (Fermentas, Lithuania). Sets of primers for μ-opioid receptor and β-actine gene amplification, as well as conditions for PCR analysis, were taken from [8].

Biodistribution

Mice with tumours over 0.5 cm in diameter were used for biodistribution studies. The course of experiment, [^{131}I]-peptide injections and results calculation were performed according to Mirowski et al [8].

Results

Accumulation of the labelled endomorphins 1 and 2 in mouse organs, tissues and tumours were investigated and compared to morphiceptin, specific μ-opioid receptor ligand [8]. These data are summarized in Tables 1 and 2. The highest endomorphin-1 saturation in mouse mammary adenocarcinoma occurred 30 minutes after administration of the ligand. Accumulation of the peptide in tumour tissue later had a tendency to decrease. Similar accumulation was noticed for morphiceptin. The kinetics of endomorphin-2 accumulation in tumour tissue was different. Its highest concentration was noticed 3–6 hours after injection. Elimination of the endomorphins with urine in comparison to morphiceptin was slower. The percentage of the peptide dose present in urine after 9 hours reached a value of about 14% for endomorphin-1 and 26% for endomorphin-2 (Table 1 and 2), while for morphiceptin it reached a value of more than 80%.

There was an increase in the tumour to muscle ratio of the peptide accumulation (T/M) with time. The highest value was reached 3 to 6 hours after injection for endomorphin-2 and morphiceptin. For endomorphin-1, this ratio reached the highest value 3 hours after injection (Figure 1).

To prove that the investigated peptides can interact with mammary adenocarcinoma cells through μ-opioid receptors, labelled peptides were incubated with membranes isolated from the cancer tissue in the presence of a cross-linking agent followed by denaturing polyacrylamide gel electrophoresis. Autoradiography has shown that [^{125}I]-peptide formed a radioactive complex with molecular weight of about 65 kDa. This complex reacted with polyclonal anti-μ-opioid receptor antibody in Western blot technique. Unbound [^{125}I]-peptide migrated in the front of the electrophoregram. The additional radioactive band was also visible on the

Table 1. Biodistribution of [^{131}I]-endomorphin-1 in mammary adenocarcinoma bearing mice (% of dose/g of tissue or blood and % of dose eliminated with urine, mean ± SD, n = 4, *p.i.*)

	Time				
	30 min	2 h	3 h	6 h	9 h
Blood	8.2 ± 0.6	7.5 ± 1.6	5.1 ± 1.8	2.7 ± 0.4	2.4 ± 0.2
Lung	2.1 ± 0.2	2.4 ± 0.3	1.7 ± 0.2	0.5 ± 0.2	0.6 ± 0.1
Liver	4.7 ± 0.2	3.5 ± 0.4	2.5 ± 0.2	1.4 ± 0.3	0.8 ± 0.3
Spleen	1.6 ± 0.1	1.0 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.1 ± 0.1
Kidney	5.0 ± 0.4	2.3 ± 0.3	1.3 ± 0.1	1.5 ± 0.1	1.1 ± 0.4
Stomach	6.8 ± 0.4	6.0 ± 1.9	6.3 ± 1.2	7.5 ± 1.2	4.9 ± 0.3
Intestine	6.9 ± 0.5	5.0 ± 1.3	5.4 ± 0.4	2.6 ± 0.6	1.1 ± 0.3
Tumour	**5.2 ± 0.3**	**3.0 ± 0.5**	**2.5 ± 0.15**	**2.0 ± 0.1**	**0.9 ± 0.1**
Muscle	1.5 ± 0.1	0.6 ± 0.1	0.33 ± 0.03	0.65 ± 0.1	0.3 ± 0.1
Brain	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.04	0.4 ± 0.1	0.4 ± 0.2
Urine (total)	19.5 ± 1.8	12.0 ± 0.4	10.9 ± 2.1	11.8 ± 2.4	13.8 ± 3.0

Table 2. Biodistribution of [^{131}I]-endomorphin-2 in mammary adenocarcinoma bearing mice (% of dose/g of tissue or blood and % of dose eliminated with urine, mean ± SD, n = 4, *p.i.*)

	Time				
	30 min	2 h	3 h	6 h	9 h
Blood	3.4 ± 0.5	3.8 ± 0.2	5.4 ± 0.3	4.4 ± 0.6	2.1 ± 0.2
Lung	0.2 ± 0.01	3.0 ± 0.2	3.1 ± 0.3	1.7 ± 0.6	1.8 ± 0.2
Liver	1.4 ± 0.2	1.6 ± 0.1	1.9 ± 0.1	1.6 ± 0.3	1.4 ± 0.1
Spleen	1.0 ± 0.06	0.7 ± 0.03	0.5 ± 0.02	0.3 ± 0.1	0.5 ± 0.0
Kidney	1.5 ± 0.2	1.5 ± 0.3	2.5 ± 0.08	1.9 ± 0.1	1.0 ± 0.2
Stomach	6.4 ± 0.3	20.4 ± 1.0	15.7 ± 1.9	12.7 ± 3.5	4.3 ± 1.
Intestine	1.5 ± 0.1	2.6 ± 0.1	1.6 ± 0.1	2.7 ± 0.1	2.3± 0.2
Tumour	**2.3 ± 0.3**	**2.8 ± 0.3**	**3.5 ± 0.3**	**3.8 ± 0.2**	**1.7± 0.1**
Muscle	0.6 ± 0.1	0.5 ± 0.04	0.3 ± 0.04	0.3 ± 0.1	0.3± 0.1
Brain	0.1 ± 0.08	0.2 ± 0.05	0.2 ± 0.04	0.2 ± 0.1	0.1± 0.1
Urine (total)	14.5 ± 2.9	17.9 ± 1.5	20.53 ± 0.8	24.5 ± 5.1	25.9 ± 4.2

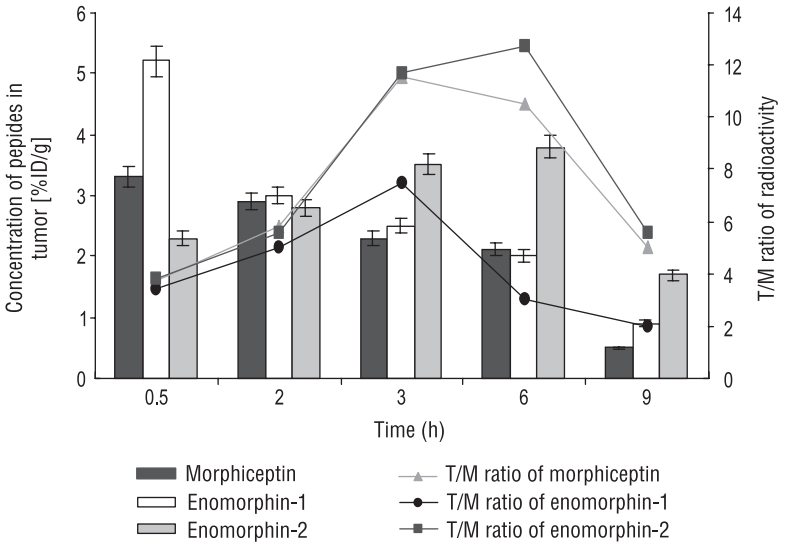

Figure 1. *In vivo* concentration (%ID/g of tumour) of [^{131}I]-endomorphin-1, [^{131}I]-endomorphin-2 and [^{131}I]-morphiceptin in mouse mammary adenocarcinoma, and tumour to muscle T/M ratio of radioactivity. Data for morphiceptin are derived from [8].

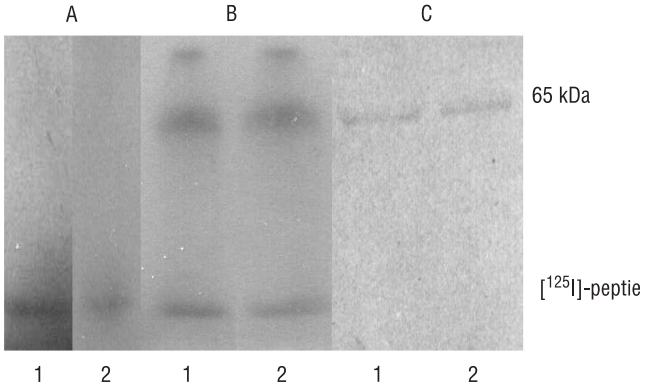

Figure 2. Autoradiographic analysis of [125I]-endomorphin-1 (1) and [125I]-endomorphin-2 (2) before (A) and after (B) incubation with the mouse mammary adenocarcinoma membranes in the presence of cross-linking reagent, Western blot analysis (C).

Table 3. Binding studies of [125I]-labelled endomorphins with membranes isolated from mammary adenocarcinoma (mean ± SD, n = 5)

Peptides	B_{max} [fmol/mg protein]	K_d [nM]
*Morphiceptin	131 ± 12	3.66 ± 0.8
Endomorphin-1	806 ± 42	0.72 ± 0.1
Endomorphin-2	671 ± 33	4.20 ± 0.1

* — data derived from [8]

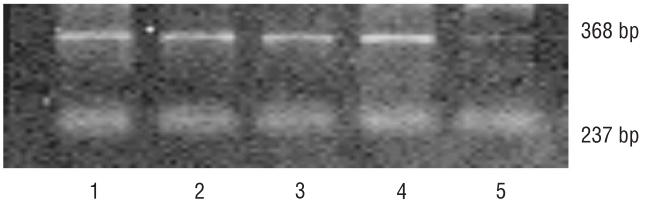

Figure 3. An example of RT-PCR analysis of μ-opioid receptor gene expression (237 bp) in mouse mammary adenocarcinoma (lanes 1–5). As a PCR control in the all analyzed cases β-actin gene (368 bp) was amplified.

border between stacking and separating gels, but this complex did not react with the antibody (Figure 2).

The binding of the [125I]-labelled endomorphins with membranes isolated from the adenocarcinoma provided information about ligand affinity (dissociation equilibrium constant K_d) and concentration of binding sites (B_{max}). K_d and B_{max} values for the studied peptides are summarized in Table 3. B_{max} for the investigated peptides has shown the significantly higher values for endomorphins in comparison to morphiceptin. The K_d value for endomorphin-1 was an order of magnitude lower than those for endomorphin-2 and morphiceptin (Table 3).

The presence of the μ-opioid receptor on mouse mammary adenocarcinoma was confirmed by RT-PCR. The analyzed tumours have shown the presence of PCR amplicon for μ-opioid receptor gene fragment (237 bp). As a PCR control, a fragment of β-actin gene (368 bp) was amplified (Figure 3).

Discussion

In several benign and malignant human and animal tumours, the specific opioid receptors were described [17]. This may suggest that they play a role in the growth and development of some tumours. The physiological relevance of the opioid receptors expression on tumour cells has been strengthened by the report that over 50% of invasive breast adenocarcinomas contain immunoreactive opioid peptides e.g. met-enkephalin, and γ- and β-endorphin [17, 18]. Specific μ-, δ- and κ-opioid receptors have been found in lung [2] and breast cancer cells [3]. Until now, however, only limited data describing the usefulness of these receptors for tumour targeting are available.

The opioid receptors have been used as a target for non-invasive radioligand methods for brain receptor studies. Various [11C]--and [18F]-compounds have been developed for labelling some of the central neuroreceptor systems [19]. The tetrapeptide morphiceptin found in enzymatic digests of casein [20] has morphine-like physiological activity and binds with high affinity to the μ-opioid receptor [21].

The above mentioned data convinced us to study the uptake of endomorphin-1 and endomorphin-2 by experimental mammary tumours. Accumulation of the endomorphins in mouse mammary adenocarcinoma was higher, and their elimination with urine lower, in comparison to morphiceptin [8]. A method based on chemical cross-linking between receptor and labelled peptide-ligands was employed because of the relatively high accumulation of endomorphins in experimental mammary tumours and their possible interaction with tumour cells through μ-opioid receptors. Such a cross-linking technique was successfully used for the detection of somatostatin receptors in human breast cancers [22] as well as for opioid receptors in rat C6 glioma cell membranes [4]. In our assay, the radioactive complex was formed between labelled endomorphins and membrane proteins of mouse mammary adenocarcinoma. Its molecular weight in SDS-PAGE was estimated at 65 kDa, which corresponded to the molecular weight of μ-opiod receptor [23]. Further analysis by Western blot technique, with use of the polyclonal anti-μ-opioid receptor antibody, provided evidence that labelled endomorphins were bound to this receptor.

Next, *in vitro* saturation studies on the binding of endomorphins to membrane proteins isolated from mouse mammary adenocarcinoma were performed. The B_{max} value for endomorphin-1 was about 800 and for endomorphin-2 about 670 fmol/mg of protein. For morphiceptin, this parameter only reached a value of about 130 fmol/mg of protein. Similar data have been described for CHO cells where μ-opioid receptor concentration was about 300 fmol/mg of protein [24]. However, K_d determined for morphiceptin and endomorphin-2 have shown nearly the same values (3.66 and 4.2 nM, respectively). This parameter for endomorphin-1 was a few times lower (0.72 nM). Similar B_{max} and K_d values were described for other μ-opioid receptor ligands. B_{max} for [3H]-DAGO for μ-opioid receptor of lung cancer cells (SCLC line H187) was determined as 175 fmol/mg protein and K_d as 5.8 nM [2]. The B_{max} value for non-SCLC line H157 was calculated as 800 fmol/mg of protein. The binding of [3H]-etorphine to MCF-7 cells has been calculated as 300–1000 fmol/mg protein (B_{max}), and the K_d value as 0.3–4 nM [3].

Additional proof, which confirmed the expression of μ-opioid receptor gene in mouse adenocarcinoma, was given by polymerase chain reaction (Figure 3). We have also demonstrated, using a radiolabelled endomorphin-2, the expression of μ-opioid receptor on protein levels in human breast cancer cells in culture (MCF-7 cell line) [25].

In the present study, we have shown a higher density of binding sites (B_{max}) in the mouse mammary adenocarcinoma for endomorphins than for morphiceptin. The highest ligand affinity (K_d) was stated for endomorphin-1. However, endomorphin-2 showed more promising characteristics in biodistribution studies. Accumulation of this peptide in tumour tissue slowly increased and reached a maximum six hours after injection. In our recent investigation, it was demonstrated that morphiceptin analogues with D-Phe or D-ClPhe in position 3 do possess a higher affinity to μ-opioid receptor than the naturally occurring morphiceptin [8]. We think, therefore, that chemical modification of endomorphin-2 structure may lead to identification of peptide(s) with a high affinity to the opioid receptors.

Results of the present study justify further investigation in this direction. The first desired step should aim at the determination of the binding capacity of endomorphin-2 and its modified analogues by human mammary tumours and normal breast tissue.

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References

1. Fimiani C, Arcuri E, Santoni A, Rialas CM, Bilfinger TV, Peter D, Salzet B, Stefano GB. μ3 opiate receptor expression in lung and lung carcinoma: ligand binding and coupling to nitric oxide release. Cancer Letters 1999; 146: 45–51.
2. Maneckjee R, Minna JD. Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines. Proc Natl Acad Sci USA 1990; 87: 3294–3298.
3. Maneckjee R, Biswas R, Vonderhaar BK. Binding of opioids to human MCF-7 breast cancer cells and their effects on growth. Cancer Res 1990; 50: 2234–2238.
4. Bohn LM, Belcheva MM, Coscia CJ. Evidence for κ- and μ-opioid receptor expression in C6 glioma cells. J Neurochemistry 1998; 70: 1819–1825.
5. Ard MD, Goldstein MN, Nash DR., Gottlieb DL. NMB: a human neuroblastoma cell line with specific opiate binding sites. Biochem Biophys Res Commun 1985; 133: 60–66.
6. Kampa M, Bakogeorgu E, Hatzoglou A, Damianaki A, Martin PM, Castanas E. Opioid alkaloids and casomorphin peptides decrease the proliferation of prostatic cancer cell lines (LNCaP, PC3 and DU145) through a partial interaction with opioid receptors. Eur J Pharmacol 1997; 335: 255–265.
7. Hatzoglou A, Bakogeorgou E, Hatzoglou C, Martin PM, Castanas E. Antiproliferative and receptor binding properties of alpha- and beta--casomorphins in the T47D human breast cancer cell line. Eur J Pharmacol 1996; 310: 217–223.
8. Mirowski M, Wiercioch R, Janecka A et al. Uptake of radiolabelled morphiceptin and its analogues by experimental mammary adenocarcinoma: *in vivo* and *in vitro* studies. Nucl. Med Biol. 2004; 31: 451–457.
9. Zadina JE, Hackler L, Ge LJ, Kastin AJ. A potent and selective endogenous agonist for the μ-opiate receptor. Nature 1997; 386: 499–502.
10. Hackler L, Zadina JE, Ge LJ, Kastin AJ. Isolation of relatively large amounts of endomorphin-1 and endomorphin-2 from human brain cortex. Peptides 1997; 18: 1635–1639.
11. Przewlocki R, Labuz D, Mika J, Przewlocka B, Tomboly C, Toth G. Pain inhibition by endomorphins. Ann NY Acad Sci 1999; 897: 152–156.
12. Asakawa A, Inui A, Momose K, Ueno N, Fujino MA, Kasuga M. Endomorphin-1, an endogenous μ-opioid receptor-selective agonist, stimulates oxygen consumption in mice. Horm Metab Res 2000; 32: 51–52.
13. Kwok EH, Dun NJ. Endomorphins decrease heart rate and blood pressure possibly by activating vagal afferents in anesthetized rats. Brain Res 1998; 803: 204–207.
14. Hatzoglou A, Ouafik L, Bakogeorgou E, Thermos K, Castanas E. Morphine cross-reacts with somatostatin receptor SSTR2 in the T47D human breast cancer cell line and decreases cell growth. Cancer Res 1995; 23: 5632–5636.
15. Greenwood FC, Hunter WM, Glower JS. The preparation of ^{131}I-labelled human growth hormones of high specific radioactivity. Biochem J 1963; 89: 114–123.
16. Mirowski M, Świtalska J, Wiercioch R, Byszewska E, Niewiadomska H, Michalska M. Uptake of radiolabelled alpha-fetoprotein (AFP) by experimental mammary adenocarcinoma and adenoma: *in vivo* and *in vitro* studies. Nucl Med Commun 2003; 24: 297–303.
17. Zagon I, McLaughlin P, Goodman S, Ehodes R. Opiod receptors and endogenous opioids in diverse human and animal cancers. J Natl Cancer Inst 1987; 79: 1059–1065.
18. Scopsi L, Balslev E, Brunner N, Poulsen HS, Andersen J, Rank F, Larsson LI. Immunoreactive opioid peptides in human breast cancer. Am J Pathol 1989; 134: 473–479.
19. Halldin C, Gulyas B, Langer O, Farde I. Brain radioligands — state of the art and new trends. Q J Nucl Med 2001; 45: 139–152.
20. Brantl V, Teschemacher H, Henschen A, Lottspeich F. Novel opioid peptides derived from casein (β-casomorphins). I. Isolation from bovine casein peptone. Hoppe Seylers Z Physiol Chem 1979; 360: 1211––1216.
21. Chang KJ, Killian A, Hazum E, Cuatreacasas P, Chang JK. Morphiceptin (NH_4-Tyr-Pro-Phe-Pro-$CONH_2$): a potent and specific agonist for morphine (μ) receptors. Science 1981; 121: 75–77.
22. Prevost G, Provost P, Salle V, Lanson M, Thomas F. A cross-linking assay allows the detection of receptors for the somatostatin analogue, lanreotide in human breast tumours. Eur J Cancer 1993; 29A: 1589–1592.
23. Liu-Chen LY, Chen C, Phillips CA. Beta-[^{3}H]funaltrexamine-labelled mu-opioid receptors: species variations in molecular mass and glycosylation by complex-type, N-linked oligosaccharides. Mol Pharmacol 1993; 44: 749–756.
24. Goldberg IE, Rossi GC, Letchworth SR, Mathis JP, Ryan-Mord J, Leventhal L, Su W, Emmel D, Bolan EA, Pasternak GW. Pharmacological characterization of Endomorphin-1 and Endomorphin-2 in mouse brain. J Pharm Exp Ther 1998; 286: 1007–1013.
25. Fichna J, Krajewska U, Różalski M, Mirowski M, Janecka A. Characterization of the [125I] endomorphin-2 binding sites in the MCF7 breast cancer cell line. Peptides 2005; 26: 395–399.