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Anti-mitogenic action of opioid peptides on epidermal growth factor-stimulated uterine cells

József L. Környei [a,*], Angéla Oszter [a], Kálmán A. Kovács [b], Zsuzsanna Vértes [a], Katalin M. Komlósi [a], Péter M. Göcze [b], Marietta Vértes [a]

[a] *Institute of Physiology, Pécs University, Medical School, Szigeti str. 12, Pécs H-7643, Hungary*
[b] *Department of Obstetrics and Gynecology, Pécs University, Medical School, Szigeti str. 12, Pécs H-7643, Hungary*

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Abstract

Endogenous opioid peptides are negative regulators of estradiol-induced uterine cell proliferation. To investigate the possible molecular target site(s) of their anti-mitogenic action, we examined the effect of opioid peptides on epidermal growth factor-induced cell proliferation both in uterine primary cell cultures prepared from adult rats and in human myometrial smooth muscle cell lines. Epidermal growth factor (EGF) significantly increased cell density in both types of cultured monolayers. This EGF-induced stimulation of cell proliferation was blocked by [D-Met^2–Pro^5]enkephalinamide in a time-dependent, receptor-mediated manner. The effective concentrations were within the physiological nanomolar range. Enkephalinamide did not have any effect on the basal rate of proliferation of the uterine cells. Our results on this novel physiological cross-talk suggest that shared step(s) of the mechanism of action of estradiol and EGF might be targeted by opioid peptides and not the general machinery of cell proliferation.

Keywords: Opioid peptides; EGF (epidermal growth factor); Uterus; Proliferation; Cross-talk

1. Introduction

The endogenous opioid peptides play an important role in the regulation of reproduction (for review, see Dyer and Bicknel, 1989). Opioid peptides (Wahlstrom et al., 1985; Bardin et al., 1987; Li et al., 1991; Zhu and Pintar, 1998), as well as opioid receptors (Baraldi et al., 1985; Vértes et al., 1986) and their mRNA messages (Jin et al., 1988; Muffly et al., 1988; Low et al., 1989; Wittert et al., 1996; Zhu and Pintar, 1998) are present in the female reproductive tract and are regulated by ovarian steroids (Baraldi et al., 1985; Wahlstrom et al., 1985; Vértes et al., 1986; Jin et al., 1988; Muffly et al., 1988; Low et al., 1989). However, their physiological role is still being investigated.

Following the original findings published by our laboratory (Vértes et al., 1982), a growing amount of data provide evidence for the inhibitory role of endogenous opioid peptides in the regulation of DNA synthesis and cell proliferation in the central nervous system (Vértes et al., 1982; Zagon and McLaughlin, 1983, 1991) and in several peripheral tissues (Maneckjee et al., 1990; Zagon et al., 1994, for review, see Zagon et al., 2000).

The endogenous opioid peptide most associated with the negative regulation of cell proliferation is [Met^5]enkephalin termed opioid growth factor. Its receptor has been cloned recently both in rat (Zagon et al., 1999) and in human (Zagon et al., 2000) tissues. Based on the nuclear localization, the different molecular structure and ligand-binding profile, the opioid growth factor receptor is different from classical opiate receptors. It is expressed in the embryonic and early postnatal phase of ontogeny and in several tumorous tissues both of neural and of peripheral origin (for review, see Zagon et al., 2000). However, no data are available on this receptor in the uterus.

Previous publications from our laboratory demonstrate that estrogen-induced DNA synthesis and cell proliferation in the rat uterus can be inhibited by opioid peptides in vivo (Ördög et al., 1992) and in vitro (Környei et al., 1997). In addition, opioid peptides have a similar action on human myometrial cells (Környei et al., 1999). In vitro estradiol is

* Corresponding author. Tel.: +36-72-536-000 ext. 1750, 1746 or 1554; fax: +36-72-536-244.
E-mail address: Jozsef.Kornyei@aok.pte.hu (J.L. Környei).

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able to stimulate uterine cell proliferation in mixed-cell types of cultures only (Környei et al., 1997, 1999), suggesting that there is paracrine signaling, possibly by peptide growth factors.

Uterine tissues and cells contain a functional epidermal growth factor/transforming growth factor-α ligand-receptor system (Lin et al., 1988; Smith et al., 1991; Rossi et al., 1992; Watson et al., 1994; Környei et al., 1995). It is accepted that estradiol acts on its nuclear receptor and induces epidermal growth factor (EGF) secretion in the uterus (DiAugustine et al., 1988; Huet-Hudson et al., 1990), and even upregulates EGF receptor levels (Mukku and Stancel, 1985; Lingham et al., 1988; Das et al., 1994; Heiner et al., 1994), thus exerting its mitogenic effect in a paracrine and autocrine manner (Nelson et al., 1991; Ignar-Trowbridge et al., 1992; Curtis et al., 1996; Galand and Rooryck, 1996; Smith, 1998). Estradiol-induced uterine tissue and organ growth needs the paracrine EGF signal from stromal cells (Ordener et al., 1993; Das et al., 1994; Hom et al., 1998). Parallel to this, peptide growth factors are able to enhance the transcriptional activity of steroid-receptor complexes (Aronica and Katzenellenbogen, 1993; Ignar-Trowbridge et al., 1993; Bunone et al., 1996; Hafner et al., 1996). Since the epidermal growth factor and estradiol mitogenic signaling systems strongly cooperate at a molecular level and even mimic each other's action (Nelson et al., 1991; Ignar-Trowbridge et al., 1992; Curtis et al., 1996; Smith, 1998), in the search for the target of endogenous opioid peptides in the uterus, it would be of interest to determine whether opioid peptides also are able to block EGF-induced, membrane receptor-mediated cell proliferation. As a first step, we have recently described that in vivo enkephalinamide implantation decreased the EGF content and, parallel to this, the DNA content of the immature rat uterus (Vértes et al., 2000).

Since no data are available in the literature, in our present experiments, we examined the in vitro direct effect of opioid peptides on epidermal growth factor-stimulated cell proliferation both in adult rat uterine primary and in human myometrial smooth muscle cell cultures.

2. Materials and methods

Uterine tissue collection, cell dispersion and culture were essentially the same as published previously (Környei et al., 1993, 1997, 1999). Here, we give a short description only.

2.1. Materials

The following chemicals and hormones were purchased from Sigma (Budapest, Hungary): Hank's Balanced Salt Solution with calcium ions and magnesium ions, Hank's Balanced Salt Solution containing no calcium ions and no magnesium ions, Trypsin-EDTA, type-XI collagenase, type-I DNase, Dulbecco's Modified Eagle's Medium (DMEM), Waymouth's medium, sodium pyruvate, minimum essential medium (MEM) amino acids, non-essential amino acids, MEM vitamins, antibiotic–antimycotic liquid, kanamycin, HEPES, sodium hydroxide, estradiol, naloxone and human recombinant epidermal growth factor. Fetal bovine serum was purchased from GIBCO BRL Life Technologies (Budapest, Hungary). The following opioid peptides were purchased from Sigma: [D-Ala^2,*N*-Me–Phe^4,Gly^5-ol]enkephalin (DAMGO), [D-Pen^{2,5}]enkephalin (DPDPE) and porcine dynorphin-A. [D-Met^2–Pro^5]enkephalinamide was a generous gift from Dr. S. Bajusz (Institute for Drug Research, Budapest, Hungary).

2.2. Tissues

Adult cycling female rats were ovariectomized, pretreated with estradiol, and then decapitated under light ether anesthesia. The uteri were removed in a sterile way. Human myometrial specimens were obtained from premenopausal women undergoing hysterectomy for benign indications with no history of hormonal treatment. The use of the tissues was approved by our institutional Human Studies Committee.

2.3. Culturing of uterine cells

Rat uterine cells were dispersed by consecutive limited enzymatic digestions with trypsin and then collagenase (Környei et al., 1997, 1999). The resulting pellets were washed, then plated to reach a final density of 10,000 viable cells (larger than 20-μm diameter)/cm^2 in 25-cm^2 Corning culture flasks, yielding about 1000 attached cells/cm^2 in 1 day. Cells were cultured in enriched Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum (GIBCO BRL Life Technologies). Experiments were performed in primary cultures.

To obtain pure human myometrial smooth muscle cell cultures, the myometrial cells were dispersed by 18 h digestion with 2 mg type-XI collagenase/ml and 0.01 mg type-I DNase/ml (Környei et al. 1993). The digests were pressed and filtered through cell-dissociation sieves. The filtrates were centrifuged, washed and plated into 150-cm^2 Corning cell-culture flasks. Attached uterine cells were cultured in Waymouth's medium containing 10% fetal bovine serum, 2% antibiotic–antimycotic solution, 1% sodium pyruvate, 2% minimum essential medium (MEM) amino acids, 1% non-essential amino acids, 1% MEM vitamins, 0.2 mg kanamycin/ml and 2% HEPES at pH 7.4 in 5% CO_2 atmosphere at 37°C. All experiments presented in this paper were performed during the first to third passages of the uterine smooth muscle cells with an initial plating density of 1000 viable cells/cm^2 in 25-cm^2 Corning culture flasks.

Culture medium was changed at 48-h intervals. Following an initial 24-h period of cell attachment, experimental treatments were present during the entire culture period. Control cultures received vehicle in the same volume.

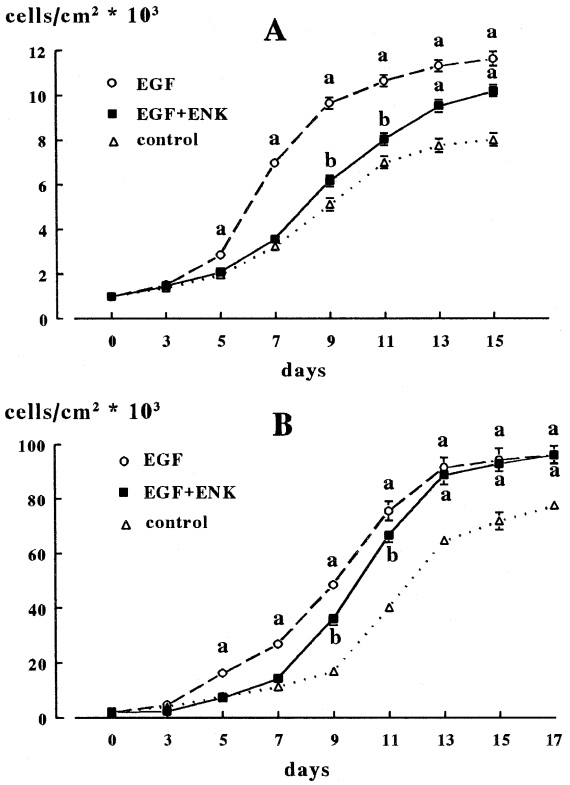

Fig. 1. Time-dependent inhibitory effect of [D-Met^2–Pro^5]enkephalinamide (ENK) on epidermal growth factor-induced (EGF) cell proliferation in rat uterine mixed-cell cultures (A) and in human myometrial smooth muscle cell cultures (B). Agents were added to the culture medium after an initial 1-day attachment period and were present in EGF 1 nM, ENK 100 nM concentrations; $n = 6$ each. Analyses of variance in panel A: $F = 0.21$, 61.43, 274.33, 303.92, 78.20, 49.43 and 41.06 for the 3, 5, 7, 9, 11, 13 and 15-day data sets, respectively, in panel B: $F = 44.84$, 285.6, 293.61, 103.62, 45.94, 23.14, 15.05 and 13.51 for the 3, 5, 7, 9, 11, 13, 15 and 17-day data sets, respectively. Post hoc Student–Newman–Keul's multiple range tests: values marked by different letters (a, b) are significantly different from the corresponding control and from each other within the data set examined at $P < 0.01$ level.

2.4. Determination of cell densities

The cell density of subconfluent cultures was determined by counting the cells, using hemocytometers. Counting was performed by two independent investigators who did not know the others' results. Parallel to this, in some pilot experiments, counting was performed in a Coulter Counter ZM as well, equipped with a 100-μm-diameter aperture tube. To further control the accuracy of our measuring techniques, in some experiments, the DNA content of one flask from the groups was determined by Burton's method (Burton, 1956).

2.5. Statistics

Figures are representatives of at least three experiments giving similar results. Not all the experiments could be performed on the same batches of cells. Data are expressed in 10^3 cells/cm^2 units, with the means and the standard errors of six data points from one representative experiment being presented. Analysis of variance followed by post hoc Student–Newman–Keul's multiple range test (Dowdy and Wearden, 1983) was used for data analysis.

3. Results

Rat uterine mixed-cell (epitheloid and myofibroblast type) cultures (panel A-s) and human myometrial smooth muscle cell lines (panel B-s) were successfully established and maintained in culture. With an average population doubling time of 1.6–2.5 days during the log phase of culture, both types of monolayers reached confluence after 10–12 days (Fig. 1A and B). After a delay of 2–3 days, 1 nM EGF treatment resulted in an 80–140% increase in cell density (Fig. 1A and B). This stimulation of cell proliferation could be significantly inhibited by the presence of 100 nM [D-Met^2–Pro^5]enkephalinamide between days 5 and 11, the density being similar to the corresponding control values on days 5–7 (Fig. 1A and B). Later, the cell cultures treated with enkephalinamide and EGF gradually lost their sensitivity for the inhibitory effect of enkephali-

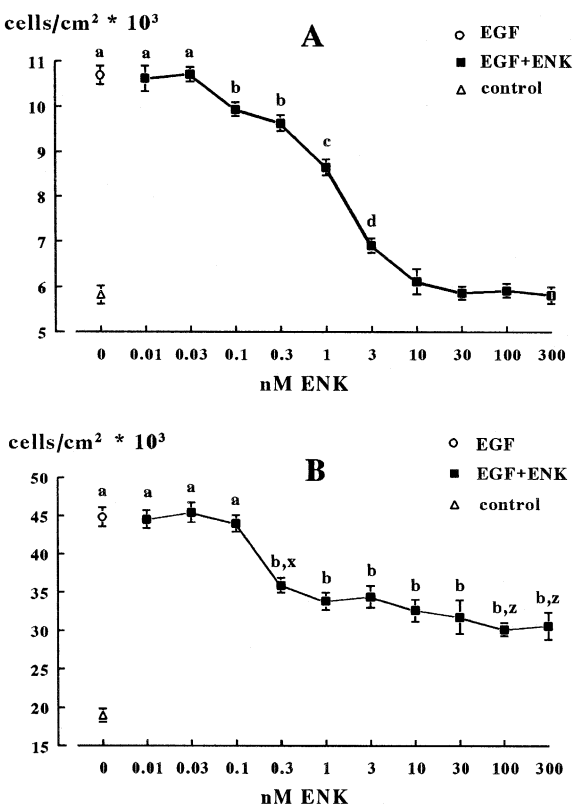

Fig. 2. Concentration-dependent inhibitory effect of ENK on EGF-induced cell proliferation in rat uterine mixed-cell cultures (A) and in human myometrial smooth muscle cell cultures (B). Treatments were present in EGF 1 nM and ENK 0.01–300 nM concentrations; $n = 6$ each. Analysis of variance in panel A: $F = 123.27$, in panel B: $F = 35.01$, post hoc Student–Newman–Keul's multiple range test: values marked by different letters (a, b, c, d in panel A and a, b, x, z in panel B) are significantly different from control and from each other at $P < 0.01$ level.

namide and were able to catch up with EGF-treated cultures.

The concentration dependence of the enkephalinamide effect (Fig. 2) revealed that the half-effective inhibitory concentration of enkephalinamide to antagonize the stimulation of cell proliferation by epidermal growth factor was in the range of 0.2–2 nM (Fig. 2A and B). No effect could be observed below 0.1 nM and complete inhibition was reached at 10 nM and higher (Fig. 2A and B). Alterations in the maximal extent of inhibition compared to stimulated and control values were due to the slightly different timing of the termination of cultures in the subconfluent phase for counting the cells (see Fig. 1).

We examined the inhibitory effect of enkephalinamide at various epidermal growth factor concentrations as well (Fig. 3). The concentration–response curve of EGF revealed that the cell density of rat uterine mixed-cell cultures (Fig. 3A) and that of human myometrial smooth muscle cell cultures (Fig. 3B) were increased by EGF in the physiological 0.05–30 nM range. The most effective concentration of EGF to stimulate uterine cell proliferation was 1 nM (Fig. 3A and B). EGF had no effect below 0.03 nM, showing a diminished effect at 2–3 nM already, and did not stimulate cell growth at 50 nM or higher (Fig. 3A and B). The presence of enkephalinamide in the culture medium completely (Fig. 3A) or partially (Fig. 3B) blocked the stimulatory effect of EGF at any EGF concentration examined (Fig. 3).

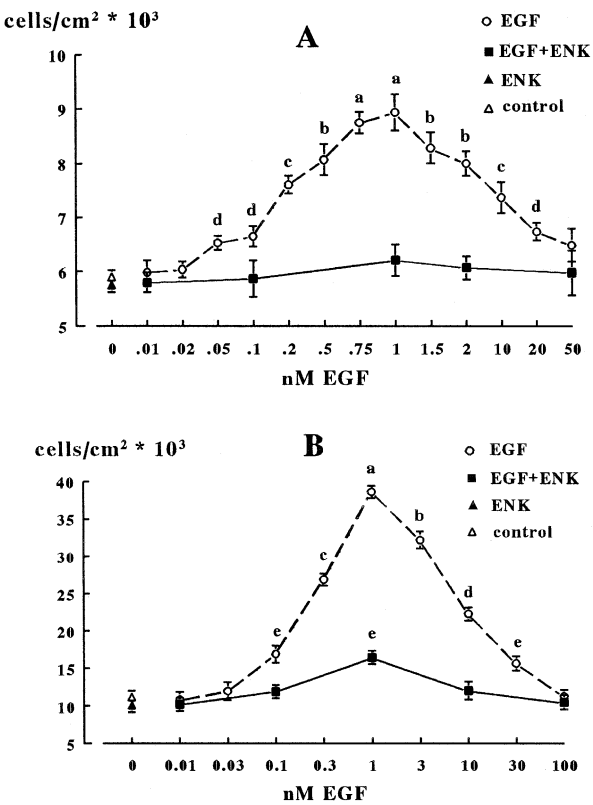

Fig. 3. Concentration dependence of the stimulatory effect of EGF on cell proliferation and its inhibition by ENK in rat uterine mixed-cell cultures (A) and in human myometrial smooth muscle cell cultures (B). Agents were present in ENK 100 nM and EGF 0.01–100 nM concentrations; $n = 6$ each. Analysis of variance in panel A: $F = 18.43$, in panel B: $F = 80.59$; post hoc Student–Newman–Keul's multiple range test: values marked by different letters (a, b, c, d, e) are significantly different from the corresponding control and from each other at $P < 0.01$ level.

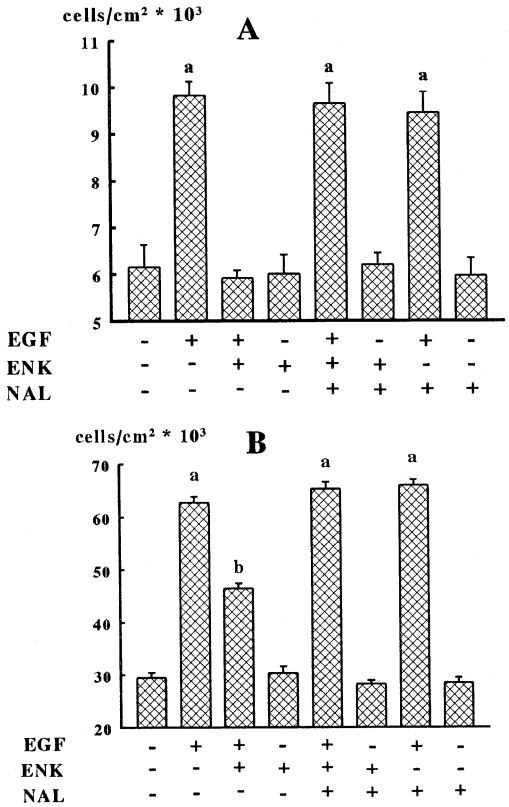

Fig. 4. Blockade of the inhibitory effect of ENK on EGF-stimulated cell proliferation by the opiate receptor antagonist naloxone (NAL) in rat uterine mixed-cell cultures (A) and in human myometrial smooth muscle cell cultures (B). The following concentrations were present (+) in the culture medium: EGF 1 nM, ENK 100 nM, NAL 100 nM; $n = 6$ each. Analysis of variance in panel A: $F = 24.05$, in panel B: $F = 292.07$; post hoc Student–Newman–Keul's multiple range test: values marked by different letters (a, b) are significantly different from control and from each other at $P < 0.01$ level.

The inhibitory effect of enkephalinamide was abolished by concomitant administration of the opiate receptor antagonist naloxone (Fig. 4A and B). EGF-stimulated cell proliferation that was inhibited by enkephalinamide. Naloxone was able to block completely the inhibitory effect of enkephalinamide. Enkephalinamide did not have any effect on basal cell proliferation. Naloxone, either alone or in combination with enkephalinamide or with EGF, did not change the cell density of rat uterine mixed cell (Fig. 4A) and human myometrial smooth muscle cell cultures (Fig. 4B).

The effect of selective opioid peptides was also studied. [D-Ala^2, *N*-Me–Phe^4,Gly^5-ol]enkephalin (DAMGO), [D-Pen^{2,5}]enkephalin (DPDPE) and dynorphin-A (100 nM each) were added to the culture medium (Fig. 5). The EGF-stimulated cell proliferation (Fig. 5, right) could be

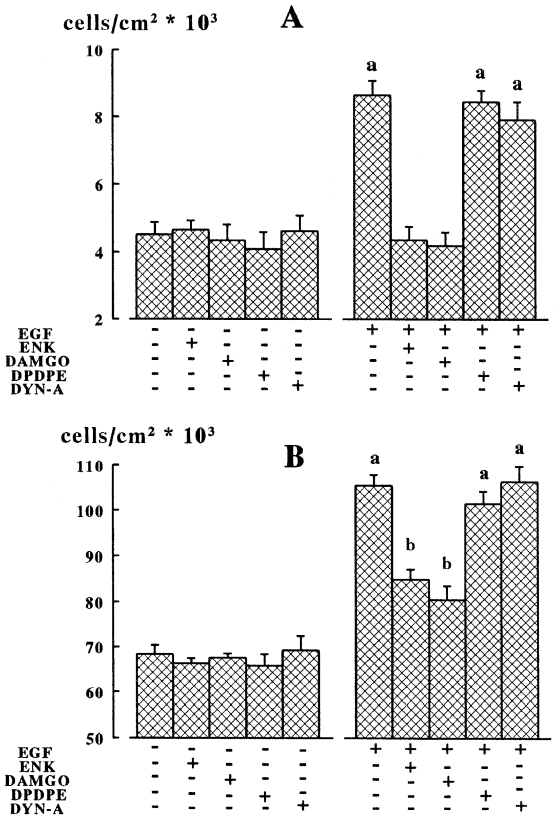

Fig. 5. Opiate receptor subtypes involved in the inhibitory effect of [D-Met^2–Pro^5]enkephalinamide (ENK) on the EGF-induced stimulation of cell proliferation in rat uterine mixed-cell cultures (A) and in human myometrial smooth muscle cellcultures (B). Cross marks: EGF 1 nM, ENK 100 nM, [D-Ala^2,*N*–Me–Phe^4,Gly^5-ol]enkephalin (DAMGO) 100 nM (μ-opioid receptor selective), [D-Pen^{2,5}]enkephalin (DPDPE) 100 nM (δ-opioid receptor selective) and dynorphin-A (DYN-A) 100 nM (κ-opioid receptor selective) opioid peptides were present. Analysis of variance in panel A: $F = 21.38$, in panel B: $F = 45.83$; post hoc Student–Newman–Keul's multiple range test: values marked by different letters (a, b) are significantly different from control and from each other at $P < 0.01$ level.

inhibited by enkephalinamide and by the μ-opioid receptor subtype-selective opioid ligand DAMGO, while the δ-specific DPDPE and κ-subtype specific dynorphin-A did not change the cell density (Fig. 5A and B). The basal proliferation of uterine cells (Fig. 5, left) was not affected by the presence of the opioid peptides administered. Our results suggest that the growth-inhibitory effect of opioid peptides might be dominantly mediated by the μ-opioid receptor in rat and human uterine cells. The results of occasionally performed parallel DNA determinations showed the same treatment-dependent differences.

4. Discussion

Data in the literature consistently show that the endogenous opioid peptides (Wahlstrom et al., 1985; Bardin et al., 1987; Li et al., 1991; Zhu and Pintar, 1998), functional opioid receptors (Baraldi et al., 1985; Vértes et al., 1986) and mRNA messages for them (Jin et al., 1988; Muffly et al., 1988; Low et al., 1989; Wittert et al., 1996; Zhu and Pintar, 1998) are present in the uterus, and their levels are regulated by ovarian steroids (Baraldi et al., 1985; Wahlstrom et al., 1985; Vértes et al., 1986; Jin et al., 1988; Muffly et al., 1988; Low et al., 1989). It is also widely accepted that endogenous opioid peptides inhibit cell proliferation within the nervous system (Vértes et al., 1982; Zagon and McLaughlin, 1983, 1991) and in several peripheral tissues (Maneckjee et al., 1990; Zagon et al., 1994; for review, see Zagon et al., 2000), including the uterus (Ördög et al., 1992; Vértes et al., 1995; Környei et al., 1997, 1999). Endogenous opioid peptides inhibit basal cell proliferation in developing rat uterus (Vértes et al., 1995; Környei et al., 1997) and in various peripheral normal and tumorous tissues and cells (Zagon et al., 1994; for review, see Zagon et al., 2000). In adult reproductive tissues, estradiol-stimulated cell proliferation is inhibited by endogenous opioid peptides (Maneckjee et al., 1990; Környei et al., 1997, 1999), whereas basal proliferation is not affected. However, the exact molecular effector site of the anti-mitogenic action of endogenous opioid peptides is still unclear.

The epidermal growth factor and estradiol mitogenic signaling systems strongly cooperate at a molecular level and even mimic each other's action in the uterus (Nelson et al., 1991; Ignar-Trowbridge et al., 1992; Curtis et al., 1996; Smith, 1998). Some data are available on the possible connections between the EGF peptide family and endogenous opioid peptide systems in the regulation of cell proliferation. Heparin-binding epidermal growth factor-like growth factor antagonizes the cell proliferation-inhibitory effect of morphine in mouse cerebellar neuroblasts (Opanashuk and Hauser, 1998), and in vivo enkephalinamide administration decreases the EGF content of the immature rat uterus (Vértes et al., 2000). In our search for the target of endogenous opioid peptides in adult uterine cells, we performed the present experiments because of the lack of in vitro data on the role of opioid peptides in the regulation of epidermal growth factor-induced cell proliferation in adult rat and human uterus.

Our data show for the first time that the endogenous opioid peptides are able to inhibit the membrane receptor-mediated, epidermal growth factor-induced proliferation of cultured rat and human uterine cells. The proliferation-inhibitory effect of enkephalinamide was found to be strictly limited in time. Significant inhibition was observed between 5 and 11 days of culture only. The downregulation of opioid receptors by prolonged agonist treatment in neural systems (Tempel et al., 1988; Ronnekleiv et al., 1996) and in rat uterus (Vértes et al., 2000) has been described already. Here, we may only suppose that long-term, high-dose enkephalinamide treatments might cause some type of downregulation or desensitization of opiate receptors or other components in the mechanism of action of endogenous opioid peptides in cultured rat uterine cells too.

As our present data show that once the inhibitory capability of enkephalinamide disappeared in about 1 week, the EGF plus enkephalinamide-treated cultures were able to accelerate cell proliferation and even reach cell densities comparable with those of vehicle-treated control cultures. However, no such catch-up phenomenon was observed previously when estradiol-stimulated cell cultures were treated with enkephalinamide (Környei et al., 1997, 1999). We suppose that EGF is so potent a stimulator of cell proliferation of uterine cells that it can even produce an overshoot effect when inhibitory effects are over or worn out. Meanwhile, it is worth noting that EGF treatment results in a smaller cell size in uterine cultures (Környei et al., 1995). Smaller cells still have time to proliferate before a confluent phase is reached and the growth of cells in monolayers is stopped by contact inhibition.

In our experiments, the effective concentrations of both enkephalinamide and EGF were within the physiological nanomolar concentration range (Li et al., 1991; Rossi et al., 1992; Környei et al., 1995). Naloxone could completely eliminate the inhibitory effect of enkephalinamide, suggesting an opiate receptor-mediated mechanism of action. Several data in the literature have demonstrated that opiate receptors are downregulated in some tissues and cells with time and by increasing concentrations of the opioid ligands used (Tempel et al., 1988; Ronnekleiv et al., 1996; Vértes et al., 2000). Our present data for uterine cells confirmed the time dependence of the effect of opiate treatment; however, no decrease in the inhibitory effect of enkephalinamide could be demonstrated at higher concentrations of the opioid peptide. Possible slight differences in the opiate mechanism of action between neural and uterine cells might explain this discrepancy.

Previously, we have also found 1 nM to be the optimal concentration of epidermal growth factor to stimulate the proliferation of human myometrial smooth muscle cells in culture (Környei et al., 1995), whereas higher concentrations are ineffective. The epidermal growth factor receptor is downregulated or internalized in a time- and concentration-dependent manner (Yanai et al., 1990; Watson et al., 1994; Környei et al., 1995; Ouyang et al., 1999). Our present results confirm previous findings where a decrease in the effect was demonstrated at higher EGF concentrations. However, endogenous opioid peptides were able to inhibit the EGF effect at all EGF concentrations examined. This contradicts in part the recent findings of Opanashuk and Hauser (1998), where heparin-binding epidermal growth factor-like growth factor antagonized the cell proliferation-inhibitory effect of morphine in mouse cerebellar neuroblasts. The difference in tissues examined (neuronal versus uterine) may contribute to this discrepancy.

Based on numerous pharmacological and molecular biology studies, it is accepted that classical opiate receptors belong to three major distinct subtypes known as μ, δ and κ (Mansour et al., 1995; Pasternak and Standifer, 1995; Satoh and Minami, 1995). As our data show, only the μ-opioid receptor-selective peptide DAMGO mimicked the action of enkephalinamide, thus the receptor subtypes involved in the proliferation-inhibitory effect are suggested to belong mainly to the μ-opioid receptor subtype. These findings are consistent with the presence of mainly μ mRNA, and less δ- and κ-opioid receptor mRNA, in the rat uterus (Wittert et al., 1996) and with the μ-preferring binding characteristics of [D-Met^2–Pro^5]enkephalinamide (Bajusz et al., 1977; Rónai et al., 1981). As the opioid growth factor receptor, which is different from classical opiate receptors, is not expressed in most normal adult tissues (for review, see Zagon et al., 2000), it was not investigated in our present experiments. However, it is worth noting, that the inhibition of basal cell proliferation in 7-day-old rat uterus (Környei et al., 1997) might be connected with the opioid growth factor receptor.

Rat uterine primary mixed-cell cultures contain roughly equal amounts of epitheloid and myofibroblast types of cells (Környei et al., 1997). The EGF and enkephalinamide treatments we used resulted in similar effects in the colonies of epitheloid and myofibroblast-type cells, as revealed by photographs taken under a phase-contrast microscope (data not shown). The relative amount of these two cell types was not changed by the treatments added. The purity of human myometrial smooth muscle cells has been documented previously (Környei et al., 1993). To compare the results, in some parallel experiments we tested human myometrial mixed-cell cultures as well (Környei et al., 1999). No difference in EGF and enkephalinamide action could be demonstrated between pure smooth muscle- and mixed-cell cultures of human myometrium (data not shown).

As the fetal bovine serum used in our experiments contains numerous biologically active molecules that might bias the observed effects, we performed some of the experiments under serum-free conditions as previously published (Környei et al., 1995). At very low cell densities, the same effect of EGF and enkephalinamide could be demonstrated (data not shown). It is worth noting that in our experimental setup, we used full-scale mitogenic stimulation and inhibitory effects were investigated.

To confirm our data on this novel regulatory cross-talk, we compared the results obtained for rat uterine mixed-cell cultures with those for human myometrial smooth muscle cell lines. Both the EGF and the enkephalinamide effects were very similar in both cell culture systems examined. Enkephalinamide inhibited the EGF-stimulated proliferation of uterine cells. The magnitude of this inhibitory effect was dependent on the timing of cell counting, rather than on the type of cell culture used. The different cell densities of the two subconfluent culture systems were due to the significant differences in the size of the attached cells.

Our novel results presented here, along with previously published data, are consistent in showing that estradiol-induced cell proliferation in adult reproductive tissues can be

inhibited by endogenous opioid peptides, while basal proliferation is not affected (Maneckjee et al., 1990; Ördög et al., 1992; Környei et al., 1997, 1999). Estradiol, when acting on its nuclear receptor, leads to EGF secretion (DiAugustine et al., 1988; Huet-Hudson et al., 1990) and to increased EGF receptor levels (Mukku and Stancel, 1985; Lingham et al., 1988; Das et al., 1994; Heiner et al., 1994), and antibodies against EGF or EGF receptors blocked estrogenic responses (Nelson et al., 1991; Ignar-Trowbridge et al., 1992; Curtis et al., 1996). With regard to the role of endogenous opioid peptides and whether they can affect EGF production or EGF receptor levels, we have recently published that the EGF content of the immature rat uterus can be decreased by enkephalinamide treatment in vivo (Vértes et al., 2000), paralleled by a decrease in the DNA content of the tissue as well. No information is available for the adult rat uterus. At present, we do not know whether the action of opioids is exerted by targeting the estrogen receptor directly or not. However, the EGF-induced phosphorylation of the estrogen receptor leads to unliganded activation of it and to full estrogenic responses (Bunone et al., 1996; Curtis et al., 1996). If both mechanisms are targeted by endogenous opioid peptides in tissues of adult animals, it might explain why both estradiol and epidermal growth factor-stimulated proliferation of uterine cells can be blocked effectively by opioid peptides. Based on these results, it seems to be unlikely that endogenous opioid peptides target general key elements in the mitogenic process of cells from adults. Opioid peptides might rather aim at elements shared by estradiol and epidermal growth factor in their mechanism of action, directing mitogenic signals from the surrounding to the cell molecular machinery. To clarify the detailed molecular mechanism of action of the novel cross-talk between the opioid, epidermal growth factor and estradiol signaling systems in uterine physiology, further experiments are required.

In summary, here, we demonstrate for the first time that endogenous opioid peptides inhibit the epidermal growth factor-induced proliferation of rat and human uterine cells in culture. Epidermal growth factor markedly increased the cell density. This cell proliferation-stimulatory effect was inhibited by enkephalinamide in a time-dependent manner. The proliferation-inhibitory action of enkephalinamide was antagonized by naloxone, while naloxone alone did not have any effect on cell density. μ-Opioid receptors mainly mediate the proliferation-inhibitory action of endogenous opioid peptides in rat and human uterine cell cultures. The observed effects were elicited by the peptides in the physiological nanomolar concentration range. Enkephalinamide did not have any effect on the basal cell proliferation of uterine cells from adult rats and from human normal myometrium. This novel cross-talk between the opioid peptide and epidermal growth factor signaling systems may play an important role in physiological regulation of the uterus during the menstrual cycle and pregnancy. Based on our present results, it can be assumed that a shared or common step(s) in the mechanism of the mitogenic action of epidermal growth factor and estradiol might be targeted by endogenous opioid peptides in uterine cells. Further studies on the mechanism of the proliferation-inhibitory role of endogenous opioid peptides may lead to a better understanding of the pathomechanism of human uterine disorders such as leiomyoma and endometrial cancer.

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