Journal of Cellular Biochemistry 73:204–211 (1999)

Opioid Agonists Modify Breast Cancer Cell Proliferation by Blocking Cells to the G_2/M Phase of the Cycle: Involvement of Cytoskeletal Elements

Simone Panagiotou,[1] Efstathia Bakogeorgou,[3] Evangelia Papakonstanti,[4] Anastassia Hatzoglou,[2,3] France Wallet,[1] Christophe Dussert,[1] Christos Stournaras,[4] Pierre-Marie Martin,[1] and Elias Castanas[3]*

[1]Laboratory of Experimental Cancerology, CJF 93–11, Marseille, F-13926 France
[2]CJF 95–02, INSERM, Clamart, F-92140 France
[3]Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, GR-71110 Greece
[4]Laboratory of Biochemistry, University of Crete, School of Medicine, Heraklion, GR-71110 Greece

Abstract Opioids decrease cell proliferation in different systems including breast, prostate, lung, kidney, and intestine, through an interaction with opioid as well as other membrane-receptor systems (somatostatin, cholinergic), through an unidentified mechanism. Recently, we have reported an interaction of taxol with opioid membrane sites (BBRC 235, 201–204, 1997), and an involvement of opioids to the modification of actin cytoskeleton in renal OK cells (J Cell Biochem. [1998] 70:60–69), indicating a possible action of the opioid effect. In the present work, we have examined the effect of two general opioid agonists (ethylketocyclazocine and etorphine) on the cell cycle, in human breast cancer T47D cells, as well as a possible modification of the cellular cytoskeleton under their action, in order to explain the antiproliferative effect of these agents. These two opioids produce a dose-dependent and reversible decrease of the proliferation of T47D cells, with a maximun attained at 10^{-8} M. The addition of 10^{-8} M of either opioid produced a significant increase of the number of cells arrested in the G_2/M phase. Confocal laser microscopy revealed a modification of the actin and tubulin microfilaments, with a clear redistribution at the periphery of the cell, reversed by the addition of the general opioid antagonist diprenorphine. Furthermore, differences between the two opioids were obvious, attributed to the different receptor affinity of each agent. The observed redistribution of actin and tubulin cytoskeletal elements gives therefore a possible answer of the antiproliferative action of opioids. The modification of the cytoskeleton, directly involved to cell division, might provoke a "mechanical" obstacle, which could be the reason of the antiproliferative effect of these agonists. Furthermore, the observed tubulin-opioid interaction by opioids provides a possible explanation of the arrest at the G_2/M phase of T47D cells under opioid treatment. Nevertheless, although the observed interaction of opioids with cytoskeletal elements gives a plausible answer of the antiproliferative effects of the agents, this might not be the only action of these agents in cell proliferation. Other, direct or indirect, genomic actions, which which remains to be elucidated, might be taken into consideration. J. Cell. Biochem. 73:204–211, 1999.

Key words: opioids; breast cancer cells (T47D); cell proliferation; cell cycle; cytoskeleton (actin, tubulin)

Previous results of our group have indicated that opioid agonists decrease cell proliferation in different systems, including the breast [Hatzoglou et al., 1996b], prostate [Kampa et al., 1997], and kidney [Hatzoglou et al., 1996c]. Nevertheless, the mechanism of action of these powerful agents is not well elucidated. Opioids act through membrane receptors, belonging to the superfamily of the seven-loop transmembrane G-protein-coupled receptors [for review, see Reisine and Bell, 1993]. Their action is proposed to be mediated by the interaction of the intracellular part of the receptor with Gi proteins. However, it was reported that, in some cases, this inhibitory action of opioids could involve other membrane receptor systems, in-

Grant sponsor: University of Crete; Grant sponsor: Greek Anticancer Society; Grant sponsor: General Secretariat of Research and Technology (GGET); Grant sponsor: Varelas SA; Grant sponsor: DELTA Dairy Products.

*Correspondence to: Elias Castanas, Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, P.O. Box 1393, Heraklion GR-71110, Greece. E-mail: castanas@med.uoc.gr

Received 31 July 1998; Accepted 14 October 1998

cluding somatostatin [Hatzoglou et al., 1995b] or cholinergic [Maneckjee and Minna, 1990, 1994].

The modification of cell proliferation by opioids could involve a specific arrest of cells in a specific phase of the cell cycle. This implies the interaction of opioids or their second-messenger system(s) with a great number of soluble and insoluble cellular elements, including specific cyclins, cyclin-dependent kinases, and a great number of intermediate products of the cell signaling machinery. On the other hand, the precise mediation of the opioid signal is unknown. Although the interaction of the opioid receptor with Gi proteins seems to be an early event of opioid action [Reisine and Bell, 1993], very little is known about the opioid-related signal propagation. Very recently, we described an interaction of opioids with actin cytoskeleton in the opposum kidney (OK) cell line [Papakonstanti et al., 1998]; we have proposed that actin cytoskeleton might mediate, at least some of the inhibitory effects of opioids [Papakonstanti et al., 1998]. Furthermore, we have described an interaction of Taxol with membrane opioid receptors in the breast, and a putative homology of the Taxol tubulin binding sites with the first extracellular loop of the opioid receptor [Bakogeorgou et al., 1997]. In view of these results, it is tentative to look about an opioid-cytoskeletal interaction.

In the present paper, we describe the effect of opioid agonists on the cell cycle. Our results indicate that, in the T47D breast cancer cell line, opioids produce a specific block of cells at the G_2/M phase, in contrast with other antimitotic agents. This effect was accompanied by a modification of the cellular cytoskeleton.

MATERIALS AND METHODS

Cell Cultures

The human breast cancer cell line T47D was obtained at passage 86. Cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM)/F12 medium, supplemented with 10% heat-inactivated fetal calf serum (FCS). The cells were cultured at 37°C, in a humidified atmosphere of 5% CO_2 in air.

Cell Growth Conditions

Cells were plated in 24-well plates at an initial density of 25×10^3 cells/well supplemented with 1 ml medium/well. All drugs were added to cultures 1 day after seeding (designated day 0), to ensure uniform attachment of cells at the onset of the experiments. Cells were grown for a total of 4 days. Opioid-containing medium was changed every 2 days. Without the addition of any drug, the proliferation time of cells was 2 days. Therefore, the number of cells in control experiments was about 100,000 at day 4. All added drugs were dissolved, in phosphate-buffered saline (PBS), shortly before use.

Cell Proliferation

Cell growth was measured by the tetrazolium salt assay [Mosmann, 1973]. Cells were incubated for 4 h at 37°C with the tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), and metabolically active cells reduced the dye to purple formazan. Dark blue crystals were dissolved with propanol. The absorbance was measured at 570 nm and compared with a standard curve of known numbers of T47D cells. All experiments were performed a minimum of three times, in triplicate.

Determination of Cell Cycle by Image Analysis

Cells were seeded and grown on 8-chamber culture slides, for 4 days, in DMEM/F12 medium, supplemented with 10% heat-inactivated FCS, without or with the addition of 10^{-8} M of opioid alkaloids, as described above. Determination of the cell cycle was made by quantitative microscopy after a Feulgen-Rossenbech stoichiometric DNA staining. For this staining, cells were fixed for10 min in water-acetone-formaldehyde (6:9:5 v/v) containing 0.02% disodium phosphate, 0.1% monopotasium phosphate, washed four times with distilled water, and air dried. Hydratation was performed by immersing slides successively in 95% and 70% ethanol and then in water for 10 min. This was followed by a 1-h hydrolysis in 6 N HCl. Cells were then rinsed four times in water for 1 min and stained for 1 h in Schiff's reagent. Then they were washed four times (1 min each) in 0.05 N HCl containing 0.026 M sodium bisulfate, then for 10 min in running water.

The image analyzer used was the SAMBA 200-cell processor (TITN Co, Alcatel, Gernoble, France). The image of each nucleus was acquired with a ×100 lens, processed in 256 densitometric levels and described with 15 parameters [Galloway, 1975; Haralick et al., 1973]. To permit analysis of the cells stained in different experiments, an adjustment routine was used

to assign the same arbitrary unit to the same DNA content. G1 peak of control cells was assigned to 2,000 arbitrary units (AU). The construction of characteristic files for each cell cycle phase (G0/G1, S, G2) was realized as described previously [Colomb et al., 1989, 1991]. Briefly, the criterion used to assign cells to each phase was the integrated optical density. We then fitted 3 gauss (G), to determine the mean, and standard deviation of each population of cells. Afterward, we made a factorial discriminant analysis, taking into account the 15 parameters computed on each cell. This analysis enabled us to determine the parameters and their functions distinguishing the three reference files. A decisional discriminant analysis determined the percentage of cells in each phase of the cycle [Wallet et al., 1996]. At least one thousand cells were analyzed per slide.

Confocal Laser Scanning Microscopy

For morphological observations by confocal laser scanning microscopy, cells were cultured for 3 days onto glass slides (22×22 mm^2). In the appropriate experiments, cells were exposed to 10^{-8} M ethylketocyclazocine or etorphine, without or with the addition of 10^{-6} M of the general opioid antagonist diprenorphine. Cell fixation and direct staining for fluorescence of microfilaments by rhodamine-phalloidin were performed as described previously [Papakonstanti et al., 1996, 1998]. The coverslips were analyzed using a confocal laser scanning module (Leica Lasertechnik, Heidelberg, Germany), attached to an inverted microscope (Zeiss IM35, Zeiss, Oberkochen, Germany), equipped with an argon-krypton ion laser. Confocal images were acquired using a 63/1.25 oil immersion objective and dedicated CLSM software (Leica Lasertechnik, Heidelberg, Germany). The image data were processed with AVS software (Advanced Visual Systems, Watham, MA) and a Silicon Graphics Indigo 2 workstation (Silicon Graphics, Mountain View, CA). Fluorescence images were optimized for brightness and contrast, transferred to a personal computer and printed on a Lexmark Optra C laser printer (Lexmark, Lexington, NY), using Corel Photo Paint 7.0 software (Corel, Ottawa, Canada).

Materials

Rhodamine-phaloidine was obtained from Molecular Probes (Eugene, OR). Secondary goat anti mouse antibodies, conjugated to fluoresceine isocyanate (FITC) were purchases from Chemicon (Temecula, CA). Antibody against α-tubulin and all other chemicals were from Sigma Chemical Co. (St. Louis, MO). EKC was a gift from Sterling-Winthrop. Diprenorphine and etorphine were from Reckit and Coleman Co. Culture media were from Gibco-BRL (Gaithersburg, MD). All other chemicals were purchased from Sigma.

RESULTS

Effect of Opioid Agonists on Cell Proliferation

As reported previously [Hatzoglou et al., 1996c], in the T47D cell line, ethylketocyclazocine and etorphine inhibited cell proliferation. Opioid inhibition of cell growth is dose dependent and reversible, in the presence of 10^{-6} M of the general opioid antagonist diprenorphine (not shown). The maximal effect of opioids was obtained at about 10^{-8} M. This concentration (10^{-8} M) was further used for the determination of the maximal effect of opioids on cell cycle.

Effect of Opioids on the Cell Cycle

Figure 1 presents the effect of opioid agonists on the cell cycle, as assayed by image analysis. As shown, opioid agonists (ethylketocyclazocine and etorphine) produce a significant increase of cells arrested at the G_2/M phase of the cycle, as compared to control cells $P < 0.001$ and $P < 0.0005$ for ethylketocyclazocine and etorphine, respectively). The increase in cells arrested to the G_2/M phase was accompanied by a concomitant decrease in the percentage of cells at the G_1 phase of the cycle. On the contrary, no significant effect of opioids on cells in S-phase was observed ($P > 0.1$). This effect provides a possible mechanism of opioid-related control of cell proliferation.

Opioids Mediate Important Microfilament Reorganization

Actin cytoskeleton (Fig. 2). The effect of opioid agonists on the dynamics of the actin cytoskeleton, was examined by confocal laser scanning microscopy. In cells cultured for 3 days in the presence of serum, but in the absence of any opioid (control) actin microfilaments were stained intensely, at about the median thickness of the cells. Staining was visible in all sections up to the surface of the cells. However, in T47D cells exposed to 10^{-8} M ethyl-

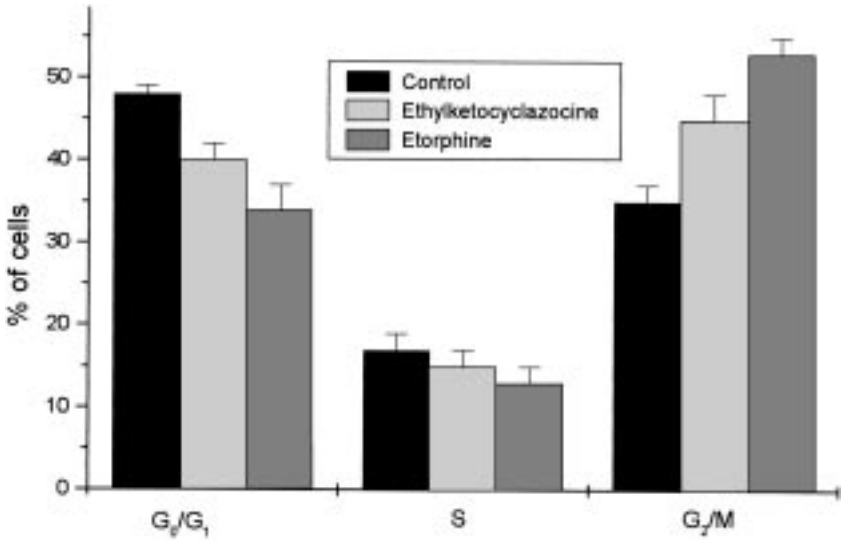

Fig. 1. Effect of opioids on the number of cells detected by image analysis in each phase of the cell cycle. Cells were grown for 4 days in 8 chamber glass slides, in the absence or the presence of 10^{-8} M ethylketocyclazocine or etorphine. Thereafter, they were stained with Feulgen-Rossenbech stoichiometric DNA staining, and analyzed by quantitative microscopy. See Materials and Methods for experimental details. Mean ±SEM of two experiments in triplicate.

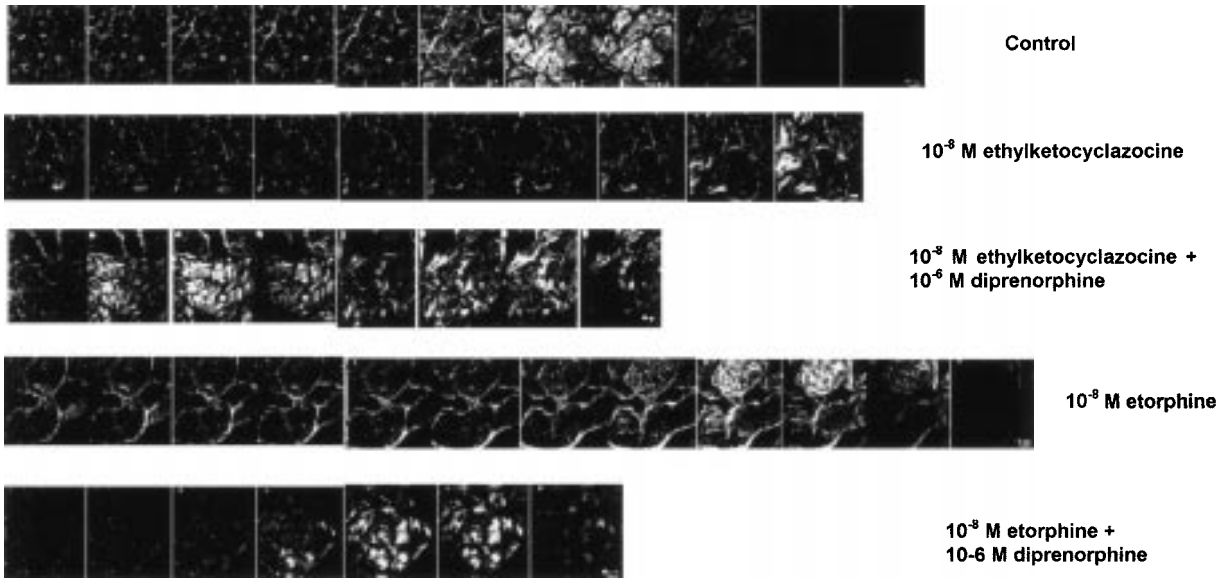

Fig. 2. Confocal laser scanning micrographs of T47D cells stained with rhodamine-phalloidin. Cells were cultured in 8-chamber glass slides, for 4 days, in Dulbecco's modified Eagle's medium (DMEM)/F12 medium, supplemented with 10% heat-inactivated fetal calf serum, in the absence (Control) or in the presence of 10^{-8} M ethylketocyclazocine or etorphine, as described under Materials and Methods. The general opioid antagonist diprenorphine (10^{-6} M) was also introduced with the respective agonists. The first section represents the upper (apical) cytoplasmic region toward the (basal) attachment site of the cells. The step size of the optical sections was adjusted to 0.5 µm. Scale bar = 10 µm.

ketocyclazocine, a clear redistribution of actin filaments was observed. At the lower scanning sections, this was characterized by intense submembranous and peripheral fluorescence, while at higher cytoplasmic scanning sections, intact microfilaments could be recognized only to a very limited extent. After etorphine treatment, actin cytoskeletal changes were more dramatic. Indeed, etorphine produced a complete reorganization of the actin microfilaments, redistributed them just below the plasma membrane. This staining was intense from the lower up to the most elevated sections. Coincubation of cells with opioids (10^{-8} M), together with 10^{-6} M diprenorphine, a general opioid agonists, reversed the effect of the opioid agonist.

Tubulin network (Fig. 3). The effects of opioids on the organization of microtubules were also studied in T47D cells. In control cells, cultured for 3 days in the absence of opioids in

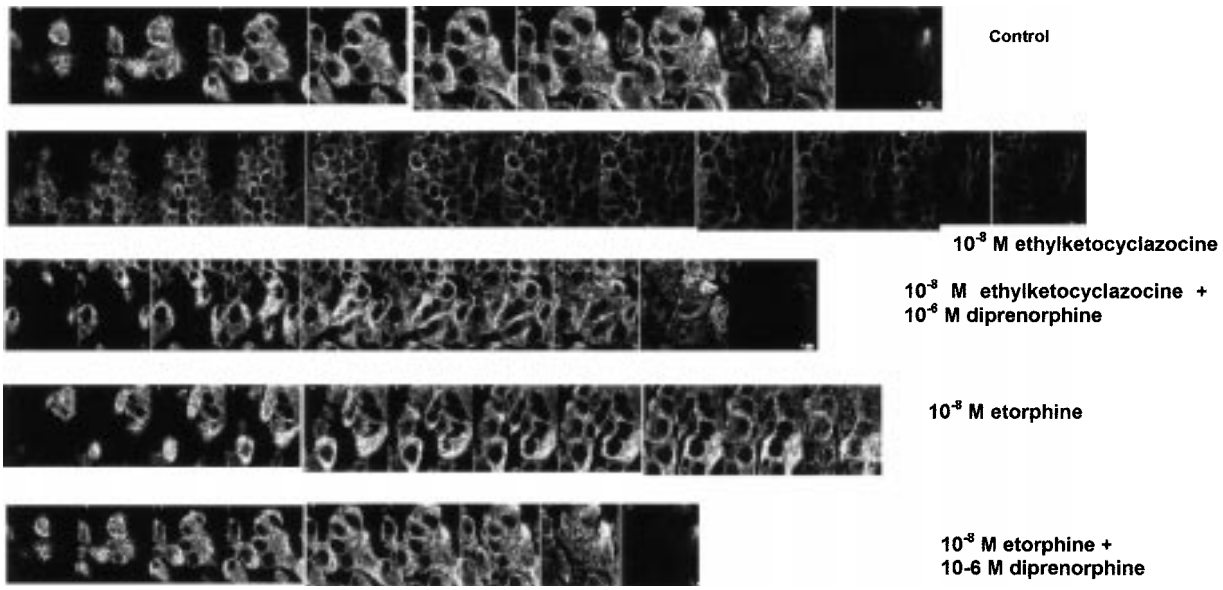

Fig. 3. Confocal laser scanning micrographs of microtubules in opioid-treated T47D cells. Cells were cultured in 8-chamber glass slides, for 4 days, in Dulbecco's modified Eagle's medium (DMEM)/F12 medium, supplemented with 10% heat-inactivated fetal calf serum, in the absence (Control) or in the presence of 10^{-8} M ethylketocyclazocine or etorphine, as described under Materials and Methods. The general opioid antagonist diprenorphine (10^{-6} M) was also introduced with the respective agonists. The first section represents the upper (apical) cytoplasmic region toward the (basal) attachment site of the cells. The step size of the optical sections was adjusted to 0.5 μm. Scale bar = 10 μm.

the same serum-containing medium, tubulin network was distributed homogeneously around the cytoplasm. On the contrary, when T47D cells were incubated with 10^{-8} M ethylketocyclazocine, the architecture of the tubulin network was profoundly modified. Indeed, in that case, the only staining that was visible was under the plasma membrane. This staining was visible in all sections, from the bottom to the surface of the cells. When diprenorphine (10^{-6} M) was applied simultaneously with EKC, the effect of the opioid was reversed. In contrast to ethylketocyclazocine, etorphine (10^{-8} M) produced a less profound alteration in the tubulin cytoskeleton. Although more intensive staining was observed under the plasma membrane, tubulin structures were also visible in all the cytoplasmic region, in all sections. In this case too, diprenorphine reversed completely the effects of the opioid.

DISCUSSION

In different cancer tissues [Levin et al., 1997; Zagon et al., 1987], and human malignant cell lines, including breast [Hatzoglou et al., 1996a; Maneckjee et al., 1990], uterus [Vertes et al., 1996], endometrium [Hatzoglou et al., 1995a], prostate [Kampa et al., 1997], kidney [Hatzoglou et al., 1996c], adrenals [Venihaki et al., 1996], intestine [Zagon et al., 1996], cornea [Zagon et al., 1995], fibroblasts [Law et al., 1997], and the central nervous system [Vertes et al., 1982; Zagon et al., 1991; Zagon and McLaughlin, 1988], opioids decrease cell growth, in a dose-dependent and reversible manner, acting through opioid receptors. In addition, opioid peptides induce also a decrease of cell proliferation of normal cardiac [McLaughlin, 1996] and adult rat uterine cells, both in vitro [Kornyei et al., 1997] and in vivo [Ordog et al., 1993; Vertes et al., 1996], in a dose-dependent manner. Although the classical means of opioid receptor action is proposed to be the inhibition of cyclic adenosine monophosphate (cAMP) accumulation through an inhibition of adelylate cyclase by receptor-coupled Gi proteins, very little is known about the postreceptor events responsible for the opioid-mediated inhibition of cell proliferation. Recently, by the construction of chimeric receptors, based on human κ opioid receptors, it was found that κ opioids provoke increased proliferation of rat-1a tissue culture cells [Coward et al., 1998]. On the contrary, β-endorphin, through the increased membrane transport of spermidine (an apoptotic signal) and polyamins in T lymphocytes, in-

creases the apoptotic death of the cells [Ientile et al., 1997], possibly explaining the antiproliferative effect of opioids. A theoretical analysis of the action of opioid alkaloids showed that opioids increase the fraction of cells in G_1/G_0 state [Glasel and Agarwal, 1997]. We have very recently described an early modification of actin cytoskeleton, 15 min after the application of opioids, persisting for at least 2 h, in opposum kidney (OK) cells [Papakonstanti et al., 1998]. Furthermore, Ca^{2+} mobilization seems to play a pivotal role in the action of opioids [Hauser et al., 1996; Sher et al., 1996].

The results of the present investigation indicate the following findings. First, opioid agonists decrease the growth of human breast cancer cells as was reported before by our laboratory [Hatzoglou et al., 1996b,c] or by other groups [Maneckjee et al., 1990]. Second, cell cycle analysis shows that cells are blocked at the G_2/M interphase, in contrast with the theoretical model presented above [Glasel and Agarwal, 1997]. Two different hypotheses could explain this latter result. The first hypothesis is that different malignant cell lines have been used in the two studies. Indeed, it is well established that cancer cell lines can express different phenotypes, concerning cyclin content and activity. In particular, the alterations to the cyclin D1 activity seem to play an important role for the alteration of the retinoblastoma pathway, which seems to be crucial for the normal tissue renewal through life [Sherr, 1996]. On the contrary, preliminary observations in T47D cells, show that the levels of immunoreactive cyclin D are not modified after a 3-day application of opioids. According to the second hypothesis, previous studies have shown that our cell lines do not possess μ opioid receptors [Hatzoglou et al., 1996a]. Indeed, our cells respond to morphine, through activation of the somatostatin receptors [Hatzoglou et al., 1995b]. Therefore, it might be plausible that activation of κ receptors lead to intracellular activation phenomena other than those triggered by the binding of morphine to the μ opioid receptor sites.

In a recent paper, we have reported that, in opposum kidney cells, κ opioids produce early alterations of the actin cytoskeletal network, in a specific and reversible manner [Papakonstanti et al., 1998]. In the present work, we extend this observation to the human breast cancer cell lines. Indeed, as shown in Figures 2 and 3, ethylketocyclazocine and etorphine, two general opioid agonists, which react with κ opioid sites in T47D cells [Hatzoglou et al., 1996b], produce important changes in both actin and tubulin network of these cells, after a 3-day application. Indeed, both microfilaments and microtubules are modified under the action of opioid agonists. They are redistributed to the periphery, under the cell membrane, leaving the cytoplasm almost depleted from cytoskeletal elements.

Although the molecular mechanism of this opioid-cytoskeletal interaction remains to be elucidated, it is plausible that GTPses [Symons, 1996] could be involved in the signaling-kinases pathway triggered by the addition of opioids. Indeed, several lines of evidences suggest that a possible mechanism controlling opioid-cytoskeletal interactions could involve the small GTPases of the Rho superfamily. Preliminary experiments from our group indicate that Rho-β GTPase is signifficantly increased in opioid-treated kidney OK cells (D. Duval and C. Stournaras, unpublished observations). A detailed study of these findings is now in progress, in an effort to establish possible signaling pathways, leading from the opioid-receptor interaction to the observed rearrangement of actin cytoskeleton and alterations of the cell proliferation.

It is interesting to note the difference in the effect of the two general opioid agonists used. Indeed, ethylketocyclazocine induces an almost complete shift of the actin network to the basal site of the cells. On the contrary, etorphine induces a submembrane redistribution of actin network. Concerning tubulin, a submembrane redistribution was the effect of ethylketocyclazocine application. In contrast, etorphine-induced effect was similar but less pronounced. Although EKC and etorphine are almost general opioid agonists, there is a clear-cut difference in their affinity on different opioid sites: EKC binds to δ, μ, κ_1, and κ_3 opioid sites, while etorphine binds with high affinity to the δ, μ, κ_2, and κ_3 opioid sites [Castanas et al., 1985a; Castanas et al., 1985b]. T47D cells possess very few δ sites, no μ receptors, and a high concentration of all three subtypes of the κ opioid site [Hatzoglou et al., 1996a]. In accord with these results, we could attribute the observed effect of EKC action on the cytoskeleton mainly to the κ_1 site, and those of etorphine to a preferential interaction with the κ_2 opioid receptor.

The observed redistribution of actin and tubulin cytoskeletal elements gives a possible answer of the antiproliferative action of opioids. Indeed, the modification of the cytoskeleton, directly involved to cell division, might provoke a "mechanical" obstacle, which could be the reason of the antiproliferative effect of these agonists.

The observed tubulin-opioid interaction by opioids gives a new insight into our previous observation that Taxol, a major anticancer drug [Rowinsky et al., 1993], with microtubule polymerization and stabilization properties [Arnal and Wade, 1995; Kumar, 1981; Nogales et al., 1995; Schiff et al., 1979; Schiff and Horwitz, 1980], can displace κ opioids from their binding sites [Bakogeorgou et al., 1997]. Indeed, the observed modifications of tubulin are similar to those observed in cells treated with Taxol. This drug and opioid alkaloids are among the rare agents that arrest the cell cycle at the G_2/M interphase.

Although the observed interaction of opioids with cytoskeletal elements provides a plausible explanation for the antiproliferative effects of the agents, this might not be the only action of these agents in cell proliferation. Indeed, we have further observed that opioid agonists modify the production of different intracellular and secreted proteins [Panagiotou et al., 1998] and further decrease the concentration of steroid receptors in the cell [Panagiotou et al., 1997]. These results imply a further, direct or indirect, effect of opioids with the cell genome, which remains to be elucidated.

REFERENCES

Arnal I, Wade RH. 1995. How does Taxol stabilize microtubules? Curr Biol 5:900–908.

Bakogeorgou E, Hatzoglou A, Castanas E. 1997. Taxol interacts with opioid binding sites in the T47D human breast cancer cell line. Biochem Biophys Res Commun 235:201–204.

Castanas E, Bourhim N, Giraud P, Boudouresque F, Cantau P, Oliver C. 1985a. Interaction of opiates with opioid binding sites in the bovine adrenal medulla. I. Interaction with delta and mu sites. J Neurochem 45:677–687.

Castanas E, Bourhim N, Giraud P, Boudouresque F, Cantau P, Oliver C. 1985b. Interaction of opiates with opioid binding sites in the bovine adrenal medulla. II. Interaction with kappa sites. J Neurochem 45:688–699.

Colomb E, Kopp F, Spyratos F, Martin P-M. 1989. Cell-cycle studies by multiparametric automatic scanning of topographically preserved cells in culture. Cytometry 10:263–272.

Colomb E, Dussert C, Martin P-M. 1991. Nuclear texture parameters as discriminant factors in cell cycle and drug sensitivity studies. Cytometry 12:15–25.

Coward P, Wada HG, Falk MS, Chan SDH, Meng F, Akil H, Conklin BR. 1998. Controlling signaling with a specifically designed Gi-coupled receptor. Proc Natl Acad Sci USA 95:352–357.

Galloway MM. 1975. Texture analysis using grey level run lengths. Computer Graphics Image Processing 4:172–179.

Glasel JA, Agarwal D. 1997. Theoretical analysis of a morphine withdrawal phenotype in a cultured cell line. Life Sci 61:PL305–313.

Haralick RM, Shanmugan K, Dinstein I. 1973. Textural feature for image classification. IEEE Trans Systems Man Cytobernetics SMC-3:610–621.

Hatzoglou A, Gravanis A, Zoumakis E, Margioris A, Castanas E. 1995a. Identification and characterization of opioid binding sites present in the Ishikawa human endometrial adenocarcinoma cell line. J Clin Endocrinol Metab 80:418–423.

Hatzoglou A, Ouafik LH, Bakogeorgou E, Thermos K, Castanas E. 1995b. Morphine cross-reacts with somatostatin receptor SSTR2 in the T47D human breast cancer cell line, and decreases cell proliferation. Cancer Res 65:5632–5636.

Hatzoglou A, Bakogeorgou E, Castanas E. 1996a. The antiproliferative effect of opioid receptor agonists on the T47D human breast cancer cell line, is partially mediated through opioid receptors. Eur J Pharmacol 296:199–207.

Hatzoglou A, Bakogeorgou E, Hatzoglou C, Martin PM, Castanas E. 1996b. Antiproliferative and receptor binding properties of alpha- and beta-casomorphins in the T47D human breast cancer cell line. Eur J Pharmacol 310:217–223.

Hatzoglou A, Bakogeorgou E, Papakonstanti E, Stournaras C, Emmanouel DS, Castanas E. 1996c. Identification and characterization of opioid and somatostatin binding sites in the opossum kidney (OK) cell line and their effect on growth. J Cell Biochem 63:410–421.

Hauser KF, Stiene-Martin A, Mattson MP, Elde RP, Ryan SE, Godleske CC. 1996. mu-Opioid receptor-induced Ca2+ mobilization and astroglial development: Morphine inhibits Dna synthesis and stimulates cellular hypertrophy through a Ca(2+)-dependent mechanism. Brain Res 720:191–203.

Ientile R, Ginoprelli T, Cannavo G, Picerno I, Piedimonte G. 1997. Effect of beta-endorphin on cell growth and cell death in human peripheral blood lymphocytes. J Neuroimmunol 80:87–92.

Kampa M, Bakogeorgou E, Hatzoglou A, Damianaki A, Martin PM, Castanas E. 1997. 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 335:255–265.

Kornyei JL, Vertes Z, Oszter A, Kovacs S, Vertes M. 1997. Opioid peptides inhibit the estradiol-induced proliferation of cultured rat uterine cells. Eur J Pharmacol 336: 65–70.

Kumar N. 1981. Taxol-induced polymerization of purified tubulin. Mechanism of action. J Biol Chem 250:10435–10441.

Law PY, McGinn TM, Campbell KM, Erickson LE, Loh HH. 1997. Agonist activation of delta-opioid receptor but not

mu-opioid receptor potentiates fetal calf serum or tyrosine kinase receptor-mediated cell proliferation in a cell-line-specific manner. Mol Pharmacol 51:152–160.

Levin RJ, Wu Y, McLaughlin PJ, Zagon IS. 1997. Expression of the opioid growth factor, [Met5]-enkephalin, and the zeta opioid receptor in head and neck squamous cell carcinoma. Laryngoscope 107:335–339.

Maneckjee R, Minna JD. 1990. Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines. Proc Natl Acad Sci USA 87:3294–3298.

Maneckjee R, Minna JD. 1994. Opioids induce while nicotine suppresses apoptosis in human lung cancer cells. Cell Growth Diff 5:1033–1040.

Maneckjee R, Biswas R, Vonderhaar BK. 1990. Binding of opioids to human MCF-7 breast cancer cells and their effects on growth. Cancer Res 50:2234–2238.

McLaughlin PJ. 1996. Regulation of DNA synthesis of myocardial and epicardial cells in developing rat heart by [Met5]enkephalin. Am J Physiol 271:R122–129.

Mosmann T. 1973. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63.

Nogales E, Wolf SG, Khan IA, Luduena RF, Downing KH. 1995. Structure of tubulin at 6.5 A and location of the Taxol binding site. Nature 375:424–427.

Ordog T, Vertes Z, Vertes M. 1993. Role of endogenous opioids in progesterone antagonism on oestradiol-induced DNA synthesis in the rat uterus. J Steroid Biochem Mol Biol 45:455–457.

Panagiotou S, Martin P-M, Castanas E. 1997. Opioid agonists decrease the concentration of steroid receptors in hormone-sensitive breast cancer cell lines. In: 2emes journees of the IFR Jean Roche. Marseille, France.

Panagiotou S, Hatzoglou A, Calvo F, Matrin P-M, Castanas E. 1998. Modulation of the estrogen-regulated proteins cathepsin D and pS2 in hormone-sensitive breast cancer cell lines (MCF7 and T47D) by opioid agonists: Evidence for an interaction between the two systems. J Cell Biochem 71:416–428.

Papakostanti EA, Emmanouel DS, Gravanis A, Stournaras C. 1996. Na^+/Pi cotransport alters rapidly cytoskeletal protein polymerization dynamics in opossum kidney cells. Biochem J 315:241–247.

Papakonstanti EA, Bakogeorgou E, Castanas E, Emmanouel DS, Hartig R, Stournaras C. 1998. Early alterations of actin cytoskeleton in OK cells by opioids. J Cell Biochem 70:60–69.

Reisine T, Bell GI. 1993. Molecular biology of opioid receptors. Trends Neurosci 16:506–510.

Rowinsky EK, Wright M, Monsarrat B, Lesser GJ, Donnerhower RC. 1993. Taxol: Pharmacology, metabolism and clinical implications. Cancer Surv 17:283–304.

Schiff PB, Horwitz SB. 1980. Taxol stabilizes micritubules in mouse fibroblast cells. Proc Natl Acad Sci USA 77:1561–1565.

Schiff PB, Fant J, Horwitz SB. 1979. Promotion of microtubule assembly in vitro by taxol. Nature 277:665–667.

Sher E, Cesare P, Codignola A, Clementi F, Tarroni P, Pollo A, Magnelli V, Carbone E. 1996. Activation of delta-opioid receptors inhibits neuronal-like calcium channels and distal steps of Ca(2+)-dependent secretion in human small-cell lung carcinoma cells. J Neurosci 16:3672–3684.

Sherr CJ. 1996. Cancer cell cycles. Science 274:1672–1677.

Symons M. 1996. Rho family GTPases: The cytoskeleton and beyond. Trends Biochem Sci 21:178–181.

Venihaki M, Gravanis A, Margioris AN. 1996. Kappa opioids exert a strong antiproliferative effect on PC12 rat pheochromocytoma cells. Peptides 17:413–419.

Vertes Z, Melegh G, Vertes M, Kovacs S. 1982. Effect of naloxone and D-met2-pro5-enkephalinamide treatment on the DNA synthesis in the developing rat brain. Life Sci 31:49–54.

Vertes Z, Kornyei JL, Kovacs S, Vertes M. 1996. Opioids regulate cell proliferation in the developing rat uterus: Effects during the period of sexual maturation. J Steroid Biochem Mol Biol 59:173–178.

Wallet F, Gerard H, Martin P-M, Dussert C. 1996. Towards a new method to in situ study of apoptosis and its relation with cell cycle. Cytometry 25:263–270.

Zagon IS, McLaughlin PJ. 1988. Endogenous opioids and the growth regulation of a neural tumor. Life Sci. 43:1313–1318.

Zagon IS, McLaughlin PJ, Goodman SR, Rhodes RE. 1987. Opioid receptors and endogenous opioids in diverse human and animal cancers. J Natl Cancer Inst 79:1059–1065.

Zagon IS, Gibo DM, McLaughlin PJ. 1991. Zeta (zeta), a growth-related opioid receptor in developing rat cerebellum: Identification and characterization. Brain Res 551: 28–35.

Zagon IS, Sassani JW, McLaughlin PJ. 1995. Opioid growth factor modulates corneal epithelial outgrowth in tissue culture. Am J Physiol 268:R942–950.

Zagon IS, Hytrek SD, McLaughlin PJ. 1996. Opioid growth factor tonically inhibits human colon cancer cell proliferation in tissue culture. Am J Physiol 271:R511–518.