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# Early Alterations of Actin Cytoskeleton in OK Cells by Opioids

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**Abstract** Recently we identified and characterized opioid binding sites in OK (opossum kidney) cells and observed decreased proliferation of these cells in response to opioids. In the present study we investigated the effects of opioids on the actin cytoskeleton and explored whether their antiproliferative action may relate to alterations in the distribution or the dynamics of actin microfilaments. Exposure of OK cells to the opioids $\alpha_{S1}$ casomorphin and ethylketocyclazocine resulted in a rapid and substantial actin microfilament reorganization. This was documented by a significant dose-dependent decrease in the amounts of F-actin, determined by measurements of quantitative fluorescence, by immunoblot analysis and by a concomitant increase of the G/total-actin ratio measured by the DNase I inhibition assay. These changes were verified by confocal laser scanning microscopy, which showed marked redistribution of the microfilamentous structures in the presence of the opioids without affecting the organization of microtubules or vimentin intermediate filaments. The effect of opioids on actin polymerization dynamics occurred within 15 min and persisted for at least 2 h, while their restoration to control levels was accomplished 6 h later, indicating a reversible phenomenon. Northern blot analysis showed that the concentration of the actin transcript was unaffected. The addition of diprenorphine, a general opioid antagonist, prevented the effects of opioids on the actin cytoskeleton. The inhibition of OK cell proliferation, induced by ethylketocyclazocine and $\alpha_{S1}$ casomorphin was partially prevented in the presence of phallacidin, which stabilizes microfilaments. Our findings demonstrate that opioids, acting via kappa 1 binding sites, induce rapidly modifications in the dynamics of actin polymerization, and in the organization of microfilaments in OK cells, which may relate to their antiproliferative effect on these cells. J. Cell. Biochem. 70:60–69, 1998. 

**Key words:** opossum kidney cells; opioid receptors; actin; microfilament reorganization; cell proliferation

Rapid changes in the dynamics of actin polymerization seem to play central roles in early cellular responses induced by a variety of stimuli, including hormones, growth factors and ions [Cantiello et al., 1991, 1993; Theodoropoulos et al., 1992; Prat et al., 1993 Fuller et al., 1994; Papakonstanti et al., 1996; Koukouritaki et al., 1996, 1997]. Diverse cellular functions such as endo- and exocytosis, secretion, cell volume regulation, membrane trafficking, and transmembrane transport seem to involve changes in the polymerization state of actin [Sontag et al., 1988; Theodoropoulos et al., 1992; Killey et al., 1992; Castellino et al., 1993; Mills et al., 1994; Mills and Mandel, 1994; Papakonstanti et al., 1996], which has been also linked to the transduction of mitogenic stimuli, through G proteins of the ras superfamily [Dartsch et al., 1994; Symons, 1996; Machesky and Hall, 1996]. In aggregate, these observations established that the actin cytoskeleton participates importantly in a variety of cellular processes, acting as a sensor and/or mediator in signal transduction [Isenberg, 1996; Gutkind and Vitale-Cross, 1996; Lim et al., 1996; Koukouritaki et al., 1997].

Exogenous and endogenous opioids have been shown to influence the proliferation of several human and animal cell culture systems [Sing-

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hal et al., 1992; Manfredi et al., 1993; Shahabi and Sharp, 1995; Zagon et al., 1996; Vertes et al., 1996; Kampa et al., 1996; Hatzoglou et al., 1996a, 1996b; Law et al., 1997]. Generally opioids act through membrane receptors, which belong to the seven transmembrane loop receptor superfamily, involving G regulatory elements and being categorized in three distinct types: delta, mu, and kappa, the latter being the most abundantly present in a variety of human and animal cells, which are further subdivided in the subtypes $\kappa_1$, $\kappa_2$, and $\kappa_3$ [Castanas et al., 1985a]. Opioid cellular actions are thought to be additionally mediated via potassium and calcium channels, whereas several opioid agonists seem to act also through somatostatin receptors [Hatzoglou et al., 1995]. The overall signaling scheme of opioid actions remain however incompletely understood, while the issue of opioid-cytoskeleton interactions has to our knowledge not been addressed to date.

Recently we have shown that OK cells posses $\kappa_1$ opioid and also somatostatin binding sites and that opioids as well as somatostatin analogs inhibit the proliferation of these cells [Hatzoglou et al., 1996c]. Since in addition these cells are equipped with a well developed cytoskeleton, which in previous work from this laboratory was shown to exhibit rapid responses to external stimulation [Papakonstanti et al., 1996], we thought it worthwhile to investigate whether neuropeptide interactions with renal epithelial cells involve alterations in actin cytoskeleton dynamics. To this end, we characterized the short-term effects of opioids on the actin cytoskeleton of OK cells and utilizing the appropriate biochemical and fluorimetric techniques we determined quantitatively the cellular concentrations of monomeric (G) and filamentous (F) actin, as well as the time and dose dependence of its (de)polymerization dynamics in response to opioids. We also followed by confocal laser scanning microscopy the morphological changes in the organization of the actin, tubulin, and intermediate filament cytoskeleton induced by opioids. Next, we examined whether the opioids induced alterations in actin polymerization dynamics are associated with changes in actin gene transcription rates and lastly we explored the potential relationship between the antiproliferative effects of opioids and actin cytoskeleton dynamics.

## MATERIALS AND METHODS

### Cell Culture

Opossum kidney cells (OK-cells) provided by the American TypeCulture Collection (Rockville, MD) were studied between passages 40 and 50. Cells were maintained in a humidified atmosphere of 5% $CO_2$—95% air in 25 $cm^2$ flasks, 24-well, or 35 mm plastic dishes at 37°C and fed twice weekly with a 1:1 DMEM-Ham's F12 medium, supplemented with 10% Fetal Calf Serum (FCS) and 2 mM glutamine, 20 mM $NaHCO_3$, 22 mM HEPES, 50 IU/ml penicillin, and 50 mg/ml streptomycine. Subcultivation was performed with $Ca^{2+}$- and $Mg^{2+}$-free phosphate buffered saline (PBS), containing 0.25% trypsin and 5 mM EDTA. Initially cells were cultured for 48 h with complete medium as described above and 15–20 h prior to the actual experiments incubations were carried out with serum-free medium containing 0.1% bovine serum albumin. All experiments were performed while cells were still in their logarithmic growth phase.

### Determination of the Cellular Monomeric (G)-Actin Levels

Monomeric (G-) and total actin content were measured using the G-actin dependent DNase I inhibition assay [Blikstad et al., 1978] with minor modifications as previously described [Papakonstanti et al., 1996]. The intracellular actin content was quantitated by reference to a standard curve of DNase I activity inhibition, prepared using rabbit muscle G-actin, isolated as previously described [Faulstich et al., 1984]. A linear relationship was observed over the range of 25–70% inhibition of DNase I activity. G- and total-actin content in OK cells was related to the total cellular protein content, measured by the method of Bradford, using the Bio-Rad protein determination kit (Bio-Rad Laboratories, Palo Alto, CA), and BSA as standard.

### Quantitative Measurements of F-Actin Amounts

Cellular content of F-actin was measured by a modification of the rhodamine-phalloidin F-actin labelling method, as described previously [Koukouritaki et al., 1996]. Fluorescence intensities were determined with a Perkin-Elmer LS 3B fluorimeter (Perkin-Elmer, Oak Brook, IL), using excitation and emission wavelengths of

550 and 580 nm respectively, and was related to the protein content of the samples, which were determined as detailed above.

### Confocal Laser Scanning Microscopy

For morphological observations by confocal laser scanning microscopy, OK cells were cultured for 24 h onto Type IV collagen covered glass slides (22 × 22 mm), so as to assure cellular attachment and orientation conditions analogous to those obtaining for renal tubular epithelia in situ. In the appropriate experiments, cells were additionally exposed for 30 min to $10^{-8}$ M ethylketocyclazocine or $\alpha_{S1}$-casomorphin. Cell fixation and direct staining for fluorescence of microfilaments by rhodamine-phalloidin as well as indirect staining for microtubules and vimentin intermediate filaments were performed as described previously [Papakonstanti et al., 1996]. The cover slips were analyzed as described recently [Koukouritaki et al., 1997], 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).

### Immunoblot Analysis

For measurements of monomeric (Triton soluble) and polymerized (Triton insoluble) actin amounts [Golenhofen et al., 1995], cells were incubated with or without $10^{-8}$ M ethylketocyclazocine or $\alpha_{S1}$-casomorphin for 15 min as described above. Cells were incubated in 500 µl of Triton-extraction buffer (0.3% Triton X-100, 5 mM Tris HCL, 2 mM EGTA, 300 mM sucrose, 400 µM PMSF, 10 µM leupeptin, 2 µM phalloidin, pH 7.4) for 5 min on ice. After removing the buffer, soluble proteins were precipitated with equal volumes of 6% PCA. The Triton-insoluble fraction remaining on the plate was precipitated with 1 ml 3% PCA. Equal volumes of each were subjected to SDS electrophoresis and the resulting protein-bands were transferred to nitrocellulose membranes, using an LKB electroblot apparatus (LKB, Bromma, Sweden). Nitrocellulose blots were incubated with monoclonal mouse anti-actin antibody, followed by incubation with the appropriate labelled secondary antibody, using the ECL Western blotting kit. Nitrocellulose blots were exposed to Kodak X-omat AR films for variable lengths of time. Band intensities were quantified by PC-based Image Analysis (Image Analysis Inc., Ontario, Canada).

### Northern Blot Analysis of Actin mRNA's

Isolation and size-fractionation of total cellular RNA from OK cells was performed as previously described [Koukouritaki et al., 1997]. Following transfer of the RNA to Gene Screen nylon membranes, the filters were prehybridized and then hybridized with cDNA probes ($\gamma$-$^{32}$P-labelled by random priming, using oligodeoxynucleotides) (specific activity $5 \times 10^7$ cpm/µg), according to Feinberg and Vogelstein, 1983. The β-actin mRNA levels were normalized to the levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Hybridizations were carried out as described [Maniatis et al., 1989]. Washed filters were exposed to Kodak XR film at -80°C in the presence of an intensifying screen. Hybridization was quantified by a PC-based Image Analyzer (Image Analysis Inc., Ontario, Canada). The probes used were a rat β-actin cDNA *Pst* I fragment inserted into a pUC 18 plasmid and a human glyceraldehyde-3-phosphate dehydrogenase cDNA probe.

### Cell Proliferation

Cells were plated in 24-well ELISA plates at an initial density of $25 \times 10^3$ cells/well supplemented with 1 ml medium/well. All drugs were added to cultures one day after seeding (designated as day 0), to ensure uniform attachment of cells at the onset of the experiments. Cells were grown for a total of 3 days, with daily changes of the medium containing drugs. All added drugs were dissolved shortly before use. 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 against a standard curve of known numbers of cells. All experiments were performed a minimum of three times, in triplicate.

### Statistical Analysis

Results are expressed as mean ± SE (n = number of cell preparations). Statistical analysis of the F- and G-actin content in treated OK-cells was performed by unpaired Student's *t*-test, pvalues less than 0.05 being considered significant.

### Materials

Culture media DMEM-Ham's F12, trypsin and EDTA solutions were from Biochrom KG (Berlin, Germany), whereas fetal calf serum was purchased from Flow Laboratories (Irving, Scotland). Bovine serum albumin, DNaseI, DNA, phallacidin, and the monoclonal antibody against α-tubulin were obtained from Sigma (St. Louis, MO). The ECL Western blotting kit was purchased from Amersham Corp. (Arlington Heights, IL). Type IV collagen was from Gibco BRL (Life Technologies, Inc., Bethesda, MD). Rhodamine-phalloidin and fluorescein-labelled goat anti-mouse IgG were from Molecular Probes Inc. (Eugene, OR). Ethylketocyclazocine was a gift from Sterling-Winthrop Co and the monoclonal antibody against vimentin was kindly provided by Dr. G. Giese (MPI, Ladenburg). Diprenorphine was purchased from Reckitt and Coleman Co. (UK), while $\alpha_{S1}$-casomorphin was synthesized as described previously [Kampa et al., 1996]. Octreotide was a gift from Sandoz Co. (Basel, Switzerland). All other chemicals were obtained from usual commercial sources at the purest grade available.

## RESULTS

### Opioids Cause Rapidly Disassembly of Microfilamentous Actin

OK cells treated with ethylketocyclazocine or $\alpha_{S1}$-casomorphin showed, within 15 min, a rapid and significant decrease of the fluorescence signal, indicating actin disassembly and reorganization, which persisted for 120 min and was restored to control levels 6 h later (Fig. 1). In contrast, treatment of the OK cells with octreotide did not affect the fluorescence signal (Fig. 1). The effects of ethylketocyclazocine and $\alpha_{S1}$-casomorphin on actin polymerization dynamics were dose dependent: As shown in Figure 2, 30 min incubation of the cells with $10^{-12}$ to $10^{-6}$ M of these opioids induced a dose dependent decrease of the fluorescence, which resulted at the highest concentration of both opioid agonists in a significant decrease by 22–25% of the F-actin levels compared to untreated controls. The effects of ethylketocyclazocine and $\alpha_{S1}$-casomorphin on actin polymerization dynamics were significantly diminished, for the time intervals described, when the cells were incubated with diprenorphine ($10^{-6}$ M) in addition to either of the two opioid agonists (Fig. 1).

### Effect of Opioids on the Dynamic Equilibrium Between Monomeric (G) and Filamentous (F) Actin and on Actin mRNA

To confirm the above findings we analyzed the ratio of monomeric to filamentous actin, i.e., the G/F actin ratio in OK cells treated with $10^{-8}$ M ethylketocyclazocine and $\alpha_{S1}$-casomorphin for various time intervals. For this, we used two distinct experimental approaches: first, the DNase I inhibition assay for G-and total actin measurements and second, quantitative immunoblot determinations of the Triton soluble and insoluble cellular actin content. Table I summarizes the results obtained with the DNase I inhibition assays. Incubation of cells with $\alpha_{S1}$-casomorphin for 30 to 120 min induced a significant increase of the G/F actin ratio, consistent with a decrease in the proportion of filamentous actin. Similar results albeit not quite reaching statistical significance were also obtained when OK cells were incubated with ethylketocyclazocine (Table I). These findings were further corroborated by quantitative immunoblot analysis of the Triton soluble (TS) and insoluble (TI) actin cytoskeleton fractions of OK cells exposed to $10^{-8}$ M opioids for 15 min (Fig. 3). As calculated from the relative band intensities, the TI/TI + TS actin ratio decreased by 21% and 15% (as percent of control, n = 2) in cells incubated with $\alpha_{S1}$-casomorphin and ethylketocyclazocine, respectively.

In order to explore whether ethylketocyclazocine and $\alpha_{S1}$-casomorphin induce changes in actin mRNA synthesis, total RNA from OK cells, incubated for 2, 4, and 6 h with the two opioid agonists or with their vehicles, was analyzed by Northern blot hybridization with labeled RNA probes complementary to actin mRNA (test) and GAPDH (control). After normalization of the actin mRNA band densities with those of GAPDH mRNA no quantitative difference was observed between opioid treated and control cells (data not shown).

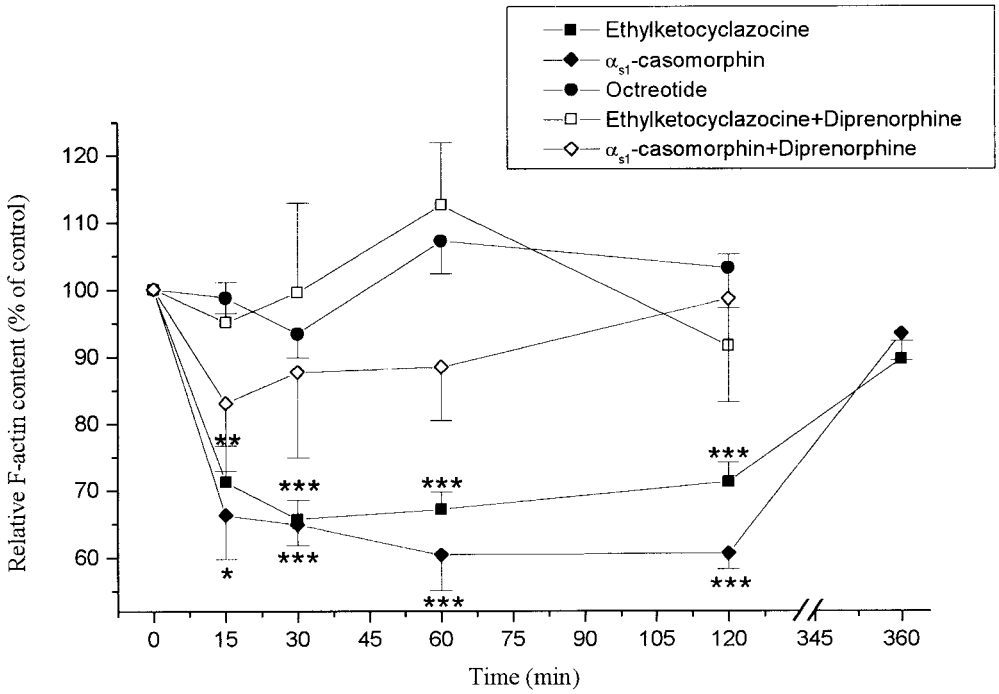


**Fig. 1.** Time dependent effects of the opioid agonists ethylketocyclazocine and $\alpha_{s1}$ casomorphin and octreotide on the F-actin content in OK cells in the absence or in the presence of the opioid antagonist diprenorphine. Cells were incubated for different time periods with $10^{-8}$ M octreotide (●), ethylketocyclazocine (■), and $\alpha_{s1}$ casomorphin (◆) or with $10^{-8}$ M ethylketocyclazocine (□) and $\alpha_{s1}$ casomorphin (◇) in the presence of $10^{-6}$ M diprenorphine. F-actin content was measured by the rhodamine-phalloidin fluorescence assay. Significance of difference vs. 100%: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, n = 4 to 10.

### Opioids Cause Marked Microfilament Redistribution Without Affecting Organization of Microtubules and Vimentin Intermediate Filaments

The rapidly mediated alterations by opioids in the dynamics of the actin cytoskeleton, shown by quantitative biochemical approaches, were further examined by confocal laser scanning microscopy. In untreated control cells actin microfilaments were stained intensely, starting from their basal attachment sites to the substratum and encompassing all sections scanned, including those of the apical cytoplasmic regions (Fig. 4A–D). However, in OK cells exposed for 30 min to $10^{-8}$ M of either ethylketocyclazocine or $\alpha_{S1}$-casomorphin there appeared a clear redistribution of actin filaments (Fig. 4E–H,I–L). 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. When cells were preincubated with diprenorphine, opioid agonists failed to induce reorganization or disassembly of the actin microfilaments (not shown), which is in agreement with the quantitative F-actin measurements (Fig. 1). In contrast to the actin cytoskeleton, microtubules and vimentin intermediate filaments were not affected following a 30 min incubation with $10^{-8}$ M ethylketocyclazocine or $\alpha_{S1}$-casomorphin, as shown by confocal laser scanning microscopy (data not shown).

### Influence of Opioids on Cell Growth and the Effects of Opioid Antagonists and of Microfilament Stabilitizing Agents

In accord with our previousy reported results [Hatzoglou et al., 1996c] ethylketocyclazocine or $\alpha_{S1}$-casomorphin inhibited proliferation of OK cells following a 4 day application (Fig. 5). Diprenorphine, as expected, largely abrogated the antiproliferative action of these opioids. Interestingly, the addition of $10^{-7}$ M of the microfilament stabilizing agent phallacidin to cells incubated in the presence of opioids restored to a significant level the growth inhibitory action of these opioids (Fig. 5). It should be mentioned that in control experiments this phallacidin concentration did not influence significantly cell growth. This finding provides experimental evidence that the actin cytoskeleton may partici-

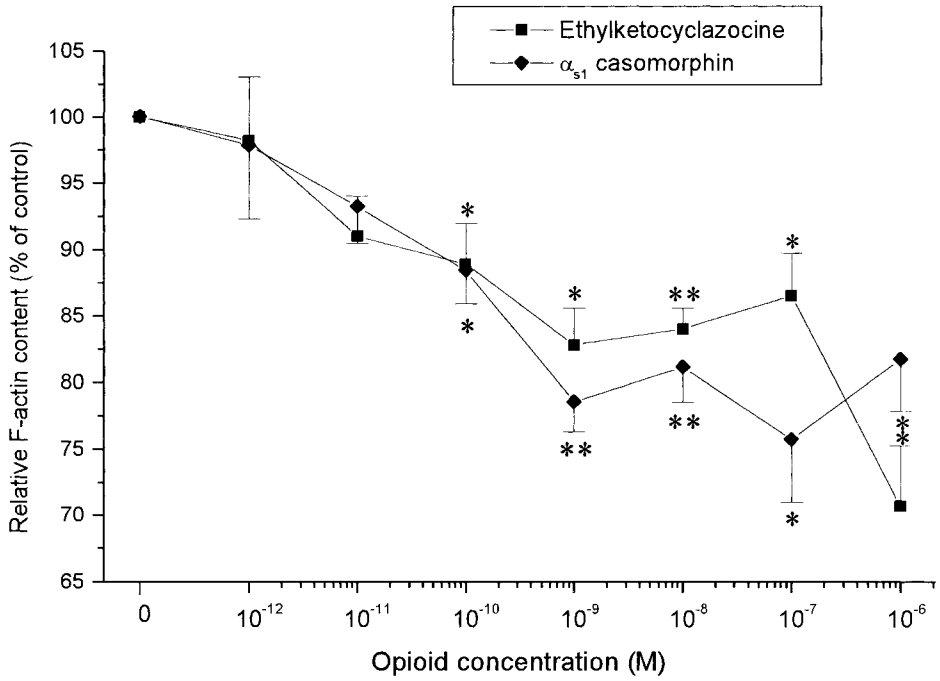


**Fig. 2.** Dose-dependent effect of ethylketocyclazocine and $\alpha_{s1}$ casomorphin on the polymerization state of actin in OK cells. Cells were incubated for 30 min with the indicated concentrations of ethylketocyclazocine (■) and $\alpha_{s1}$ casomorphin (◆). F-actin content was measured by the rhodamine-phalloidin fluorescence assay. Significance of difference vs 100%: $^{*}P < 0.05$; $^{**}P < 0.01$, n = 4.

**TABLE I. Effect of $\alpha_{s1}$ Casomorphin and Ethylketocyclazocine on the G/F Actin Ratio[a]**

| Incubation time | G/F-actin ratio | |
|---|---|---|
| | $\alpha_{s1}$ casomorphin | Ethylketocyclazocine |
| Control | 0.358 ± 0.02 | 0.396 ± 0.06 |
| 30 min | 0.444 ± 0.04* | 0.491 ± 0.07 |
| 60 min | 0.412 ± 0.01* | 0.450 ± 0.05 |
| 120 min | 0.438 ± 0.01* | 0.438 ± 0.04 |
| 360 min | 0.401 ± 0.02 | 0.419 ± 0.05 |

[a]Monomeric (G) to polymerized (F) actin ratio measured by the DNase I inhibition assay in OK cells incubated for different time periods with $10^{-8}$ M $\alpha_{s1}$ casomorphin or ethylketocyclazocine. (Significantly different from control: $^{*}P < 0.05$, n = 4).

pate in opioid agonists action on the growth of OK cells.

## DISCUSSION

Cytoskeletal elements are central to many cellular functions and their polymerization dynamics are subject to fine regulatory control [Mitchison and Cramer, 1996; Lauffenburger and Horwitz, 1996]. A number of recent studies provided evidence that transduction of external signals, in addition to classical receptor mediated pathways, may be also accomplished through interactions with the actin microfilamentous network underlying the plasma membrane [Isenberg, 1996; Lim et al., 1996; Koukouritaki et al., 1997]. Regulation of actin assembly, microfilaments' reorganization and conformational changes were identified as possible mechanisms and relationships between extracellular stimuli and modifications of the cytoskeleton are increasingly recognized as participating in the incitement of important cellular responses [Isenberg, 1996]. Opioid agonists, on the other hand, were found to affect cell proliferation in many epithelial cell lines, including OK cells via opioid and/or somatostatin receptors [Hatzoglou et al., 1996c]. However, interactions of opioids with cytoskeletal elements have not been studied to date.

Addressing the latter issue, we examined whether the cytoskeleton responds to opioids in OK cells. We selected this cell line because in our hands OK cells were proven valuable for the study of rapid alterations in the organization and dynamics of cytoskeletal structures [Papakonstanti et al., 1996], while simultaneously expressing functional opioid binding sites [Hatzoglou et al., 1996c]. Our results dem-

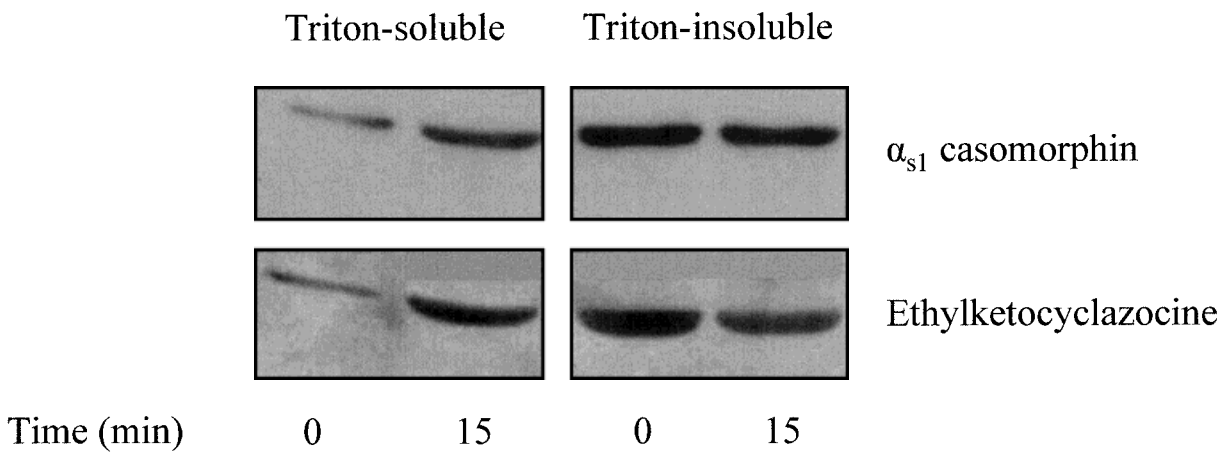


**Fig. 3.** Quantitative immunoblot analysis of actin cytoskeletal fractions in the presence of opioids. Quantitative immunoblot analysis depicting the effects of $\alpha_{s1}$ casomorphin and ethylketocyclazocine on the Triton soluble and Triton insoluble actin cytoskeleton preparations. OK cells were incubated for 15 min with $10^{-8}$ M of either of the two opioids.

onstrate important modifications of the actin-based cytoskeleton induced by two opioids, ethylketocyclazocine and $\alpha_{S1}$-casomorphin. The former, is a generally acting opioid agonist in different systems, including the breast (Hatzoglou et al., 1996a; Castanas et al., 1985a,b], which in the OK cell line however, exhibits only one high affinity opioid site of kappa1 selectivity [Hatzoglou et al., 1996c]. On the other hand, $\alpha_{S1}$-casomorphin is a human peptide, recently synthesized by our group, and derived from human $\alpha_{S1}$-casein [Kampa et al., 1996]. This agonist was found to be a very potent opioid in inhibiting cell proliferation in breast and prostate cell lines.

The cytoskeleton changes observed consist of rapid time- and dose-dependent decrement of the cellular F-actin levels and increase of the G/F actin ratio. Relevant data were acquired utilizing three appropriate and accurate, but independent, quantitative techniques, i.e., the sensitive rhodamine-phalloidin fluorimetric assay for F-actin quantitation, the DNase I inhibition assay for G- and total actin determinations and lastly, immunoblot analysis for the quantitative determination of the Triton soluble and insoluble actin cellular content. Alterations in the polymerization state of the actin cytoskeleton were detectable very early, whereas restoration of actin polymerization dynamics to control levels was accomplished 6 h later, indicating a transient and reversible phenomenon. In line with this, the transcription rate of the actin gene was not affected during this period, indicating that the opioid induced rapid and transient actin depolymerization and redistribution were distinct phenomena, which did not require actin gene transcription or actin biosynthesis. This is in line with recently reported results [Koukouritaki et al., 1997]. The modifications in cytoskeletal organisation were further studied by confocal laser scanning microscopy. In response to opioids, microfilaments seem to depolymerize at the apical sections, while enhanced fluorescence became evident at the basal and peripheral cytoplasm. Accordingly, both biochemical data and morphological observations indicate clearly that the two opioids studied induced rapidly marked rearrangement in the microfilamentous actin network. It is noteworthy, on the other hand, that confocal laser scanning microscopy failed to disclose alterations of the microtubular structures and vimentin intermediate filaments in response to opioids. We interpret these data as indicative of a direct and rapid involvement of the actin- but not tubulin and vimentin-based cytoskeleton in the response of OK cells to opioids.

The effects of ethylketocyclazocine and $\alpha_{S1}$ casomorphin causing rapid actin disassembly and redistribution appear to be specific, and mediated via interaction with kappa 1 opioid binding sites. This was suggested by: 1) that kappa 1 sites were the only opioid receptors identified on this cell line [Hatzoglou et al., 1996c], and 2) that diprenorphine (a general opioid antagonist), prevented completely the effects of opioids on cell proliferation, F-actin

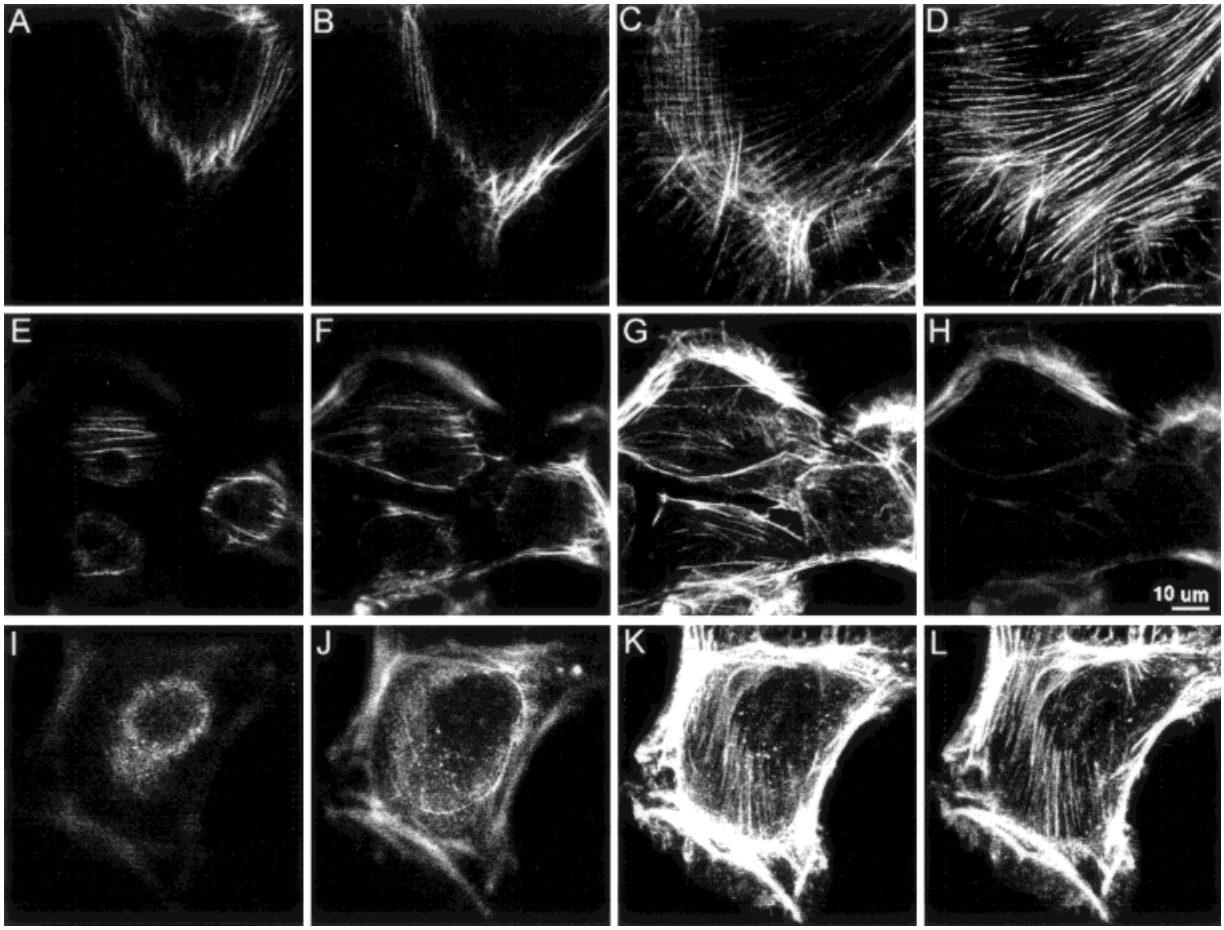


**Fig. 4.** Confocal laser scanning micrographs of OK cells stained with rhodamine-phalloidin. **A–D**: Untreated control cells. **E–H**: Cells treated for 30 min with $10^{-8}$ M ethylketocyclazocine. **I–L**: Cells treated for 30 min with $10^{-8}$ M $\alpha_{s1}$ casomorphin. A,E,I to D,H,L respectively represent scanning sections from the upper (apical) cytoplasmic region towards the (basal) attachment site of the cells. The step size of the optical sections was adjusted to 0.5 µm. Scale bar = 10 µm.

fluorescence, and reorganization of microfilaments. Moreover, the involvement of the kappa1 opioid binding site in the redistribution of actin microfilaments, is further supported by experiments in which the influence of octreotide was studied on actin polymerization dynamics. As shown in Figure 1, this somatostatin analogue, which interacts with OK cells via somatostatin binding sites, distinct from those for opioids with kappa 1 selectivity, predictably failed to affect actin polymerization dynamics.

The molecular mechanisms by which opioids affect the actin cytoskeleton remain unclear. However, interactions of opioids with their respective cellular receptors are believed to involve GTP-binding proteins [Reisine and Bell, 1993]. Since recently a pathway linking the Ras-related family of Rho GTPases to the regulation of actin cytoskeleton and signalling kinase cascades has been recognized [Dartsch et al., 1994; Symons, 1996], it is conceivable that activation by opioids of small GTP-binding proteins may participate in the alteration in actin cytoskeleton dynamics observed in our experiments.

The alterations in actin cytoskeleton dynamics upon opioid action indicate that modifications in actin polymerization may be involved in the reception of opioid signals, the microfilaments acting as sensors and/or mediators of these effects. Whereas, additional studies are needed to establish the biological relevance of the observed rapid opioid-actin cytoskeleton interactions which prevents microfilament depolymerization. It is interesting to note that the antiproliferative action of the two opioids studied was prevented by the addition to the incubation medium of phallacidin, an actin cytoskel-

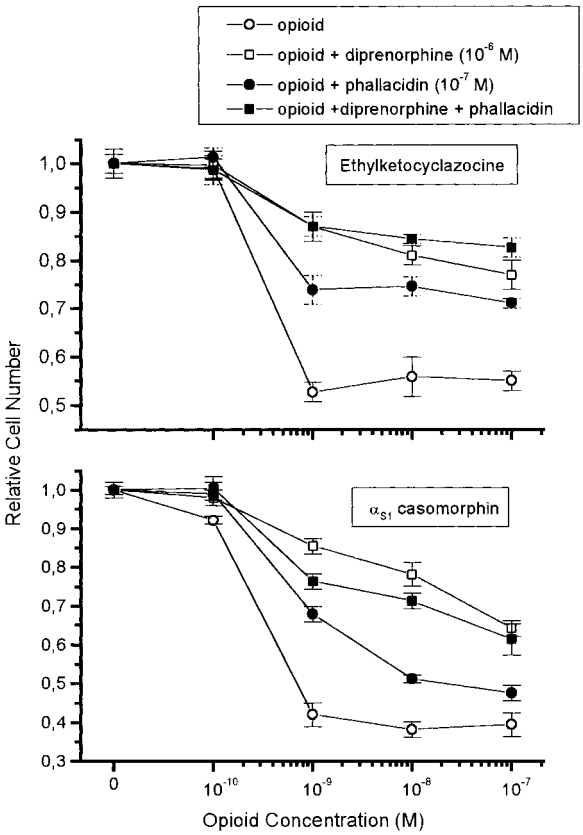


**Fig. 5.** Effects of ethylketocyclazocine and $\alpha_{S1}$ casomorphin on the proliferation of OK cells in the presence of phallacidin. Cells were incubated for 4 days, with the indicated concentrations of opioids in the absence (○) or presence (□) of $10^{-6}$ M of the general opioid antagonist diprenorphine. The same experiments were conducted in the presence of $10^{-7}$ M phallacidin with (■) and without (●) diprenorphine. Data presented correspond to the number of cells in each condition as a relative fraction of control growth, which amounted to 70,000 cells in the absence of phallacidin and 55,000 cells in its presence. Mean ± SE of two distinct experiments in triplicate.

eton stabilizer, suggesting participation of the actin cytoskeleton in the mediating effects of opioids on cell growth.

## ACKNOWLEDGMENTS

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