LABORATORY INVESTIGATIONS

Anesthesiology
1997; 87:1118–26

Opioid Effects on Mitogen-activated Protein Kinase Signaling Cascades

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Background: **The molecular mechanisms underlying both beneficial and undesirable opioid actions are poorly understood. Recently, the three currently known mammalian mitogen-activated protein kinase (MAPK) signaling cascades (extracellular signal-related kinase [ERK], stress-activated protein kinase, and p38 kinase) were shown to play important roles in transducing receptor-mediated signaling processes.**

Methods: **To determine whether any of these kinase cascades were activated by opioids, mu, delta, or kappa opioid receptors were transiently introduced into COS-7 cells together with MAPKs tagged to allow recognition by specific antibodies, and then exposed to opioids. Mitogen-activated protein kinase activation was determined by an in vitro MAPK activation assay. In addition, C6 glioma cells with either mu, delta, or kappa receptors stably introduced were exposed to opioids and MAPK activation determined by in vitro activation assay or antibody detection of activated forms.**

Results: **Transient experiments in COS cells revealed potent stimulation of ERK by mu and delta receptor activation, weak stimulation of stress-activated protein kinase by all receptor types, and no activation of p38. In stably transfected C6 glioma cells, only ERK activation was observed. Extracellular signal-related kinase induction was rapid, peaking 5 min after stimulation, and its activation was receptor-type specific. Mu and delta receptor stimulation activated ERK, but kappa stimulation did not.**

Conclusions: **These results show that acute opioid signaling is not only inhibitory, but can strongly activate an important signaling cascade. Extracellular signal-related kinase activation may contribute to desirable responses to opioids, such as analgesia and sedation, and also to undesirable adaptive responses, such as tolerance, physical dependence, and possibly addiction. Further study of this system could provide greater insight into the molecular mechanisms underlying these clinical problems. (Key words: Dependence. Extracellular signal-related kinase. Opiates. Opioid receptor. p38 mitogen-activated protein kinase. Signal transduction. Stress-activated protein kinase. Tolerance.)**

This article is accompanied by an Editorial View. Please see: Maze M: "Exciting" aspects of opiate receptor signal transduction. ANESTHESIOLOGY 1997; 87:1032–3.

OPIOIDS are the most important class of drugs used in pain treatment. However, in addition to their desirable analgesic properties, opioids have many undesirable side effects, such as the development of tolerance and the risk of addiction. Despite many recent advances, the molecular mechanisms responsible for acute opioid effects and long-term behavioral adaptations are incompletely understood. It is known that opioid receptors are members of the G-protein coupled receptor family. They generally couple to G_i and G_o classes of G proteins and acutely inhibit cyclic adenosine monophosphate formation, inhibit calcium conductance, and activate a potassium conductance, leading to cell hyperpolarization.[1] Although these changes account for part of the opioid signaling response, the picture is far from complete. Despite the similarities in signal transduction mechanisms, the effects caused by the opioid subtypes are different. For example, mu and delta opioid agonists can produce euphoria and are positive reinforcers,

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Received from the Ontario Cancer Institute, Toronto, Ontario, Canada. Submitted for publication December 20, 1996. Accepted for publication June 9, 1997. Supported by National Institutes of Health grant DA 00223 (to H.B.G.), DA 02265 and 08920 (to H.A.), and MRC Canada (to J.R.W.). Results were presented at the International Narcotics Research Conference, Long Beach, California, July 21, 1996, and at the American Society of Anesthesiologists 1996 annual meeting, New Orleans, Louisiana, October 22, 1996.

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whereas kappa agonists cause dysphoria and are negative reinforcers.[2] It is unclear whether these properties can be completely explained by anatomic differences in the localization of different opioid receptor types, suggesting that other signal transduction mechanisms may be involved in mediating opioid effects.

In an effort to better understand the signaling pathways underlying opioid effects, researchers have begun to focus their studies on the interactions of opioids with protein kinases and their functional significance. Kinases are enzymes that phosphorylate target proteins and change their biological activity. Kinases are involved in regulating nearly all physiologic functions. It is known that chronic administration of opioids increases adenylylate cyclase levels above baseline.[3] The resultant protein kinase A activation leads to changes in the phosphorylation state of proteins relevant to opioid signaling[3,4] and alters the expression of genes regulated by protein kinase A, which may be important in the development of opioid dependence.[5] Opioids can also activate protein kinase C,[6] which can phosphorylate and desensitize the opioid receptor[7] and lead to other adaptive responses, such as activation of the N-methyl-D-aspartate receptor,[6] which could mediate the development of narcotic tolerance and dependence.[8]

In the past few years, protein kinase signaling cascades have been identified as important mediators of signaling responses in all eukaryotic organisms.[9,10] The extracellular signal-regulated kinase (ERK) cascade was the first mitogen-activated protein kinase (MAPK) system characterized in mammalian cells.[11] This cascade has been shown to have roles in development and in adaptive responses of postmitotic cells.[10] At the core of this cascade and recently identified homologous cascades is a module of three protein kinases that phosphorylate each other sequentially: a MAPK kinase kinase, an MAPK kinase, and finally an MAPK homolog (figure 1).[12] These cascades couple extracellular signals to long-term changes in cellular function by phosphorylation of both cytoplasmic and nuclear proteins by the MAPK homolog. They can also induce changes in gene expression by phosphorylating nuclear transcription factors.[13]

Recently, two additional MAPK-related kinase cascades were described in mammalian cells: the stress-activated protein kinase (SAPK) and p38 cascades (figure 1).[14,15] These pathways are activated by a wide variety of cellular stressors (heat shock, ultraviolet irradiation, and ischemia and reperfusion), inflammatory cytokines, and protein synthesis inhibitors.[9] The physiologic roles of these two cascades are not as extensively defined as they are for the ERK cascade. However, there is evidence to suggest that the SAPK cascade is an important component of apoptotic responses, whereas the p38 cascade plays an important role in regulating inflammatory responses by mediating the synthesis of certain cytokines.[16]

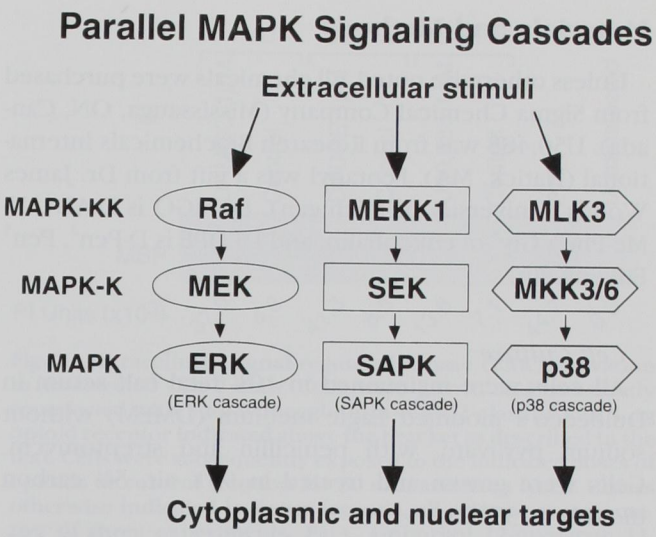


Fig. 1. Parallel MAPK signaling cascades in mammalian cells. Extracellular stimui lead to activation of MAPK cascades, which in turn leads to phosphorylation of various cytoplasmic and nuclear targets and alteration of their biological activity.

Previous findings suggested that kinase cascades could be involved in opioid signaling. Several G-protein–coupled receptors signal through the ERK and SAPK cascades. For example, the G_i-coupled receptors for thrombin, lysophosphatidic acid, and the M2 muscarinic receptor all activate ERK.[17] In addition, the M2 receptor can activate the SAPK cascade.[18] Morphine also induces c-fos expression in several brain regions.[19] Extracellular signal-regulated kinase activation has been shown to induce transcription of c-fos *via* Elk-1 phosphorylation and subsequent serum response element activation.[20] Recent in vitro and in vivo studies have also shown that ERK can be activated by opioids. In this study, we wanted to determine whether any other of the three cascades could be activated by opioids, and if the characteristics of any observed activation could be relevant to the physiologic or side effects of opioids.

Materials and Methods

Unless otherwise noted, all chemicals were purchased from Sigma Chemical Company (Mississauga, ON, Canada). U50,488 was from Research Biochemicals International (Natick, MA). Fentanyl was a gift from Dr. James Woods (University of Michigan). DAMGO is D-Ala^2, N-Me-Phe^4, Gly^5-ol enkephalin; and DPDPE is D-Pen^2, Pen^5 Enkephalin.

Cell Culture

All cells were maintained in 10% fetal calf serum in Dulbecco's modified Eagle medium (DMEM) without sodium pyruvate, with penicillin and streptomycin. Cells were grown and treated in 95% air, 5% carbon dioxide at 37°C.

COS-7 cells (derived from African green monkey kidney) were used for transient transfection experiments with rat clones of the mu, delta, and kappa receptor.[21,22] Rat-derived C6 glioma cell lines stably expressing either the mu and delta (rat) or the kappa (human) opioid receptor were developed by introduction (transfection) of receptors subcloned into pCMV vector[21,22] into wild-type C6 cells by the calcium-phosphate method,[23] followed by selection in G418-containing medium (DMEM plus 10% fetal calf serum). Receptor densities in these cells were approximately 2 pm/mg protein for mu, 1 pm/mg protein for kappa, and 0.5 pm/mg protein for delta. Ligand binding affinities for the receptors was verified (Mansour et al., manuscript in preparation).[24] Cells were maintained in media by adding 1 mg/ml G418. Experiments were performed on stable cells before passage 25.

COS-7 transient transfections were performed at 50–70% confluence in 35-mm plates with 8 μg total DNA/plate, using calcium-phosphate precipitation. Cells were plated 24 h before transfection, then treated with a 1:1 mix of 250 mM calcium phosphate and transfection buffer (136 mM NaCl, 5 mM KCl, 11.2 mM glucose, 42 mM HEPES [*p*H 7.5], 1.4 mM Na_2HPO_4 [*p*H 7.1] for 20 min. Media was then added and changed 24 h later. Opioid receptor constructs as described previously and ERK-2 (in pMT3), p46βSAPK (in pMT2), and p38 (in pCDNA3; courtesy of Dr. Brent Zanke, Ontario Cancer Institute) were used for transfections. These MAPK constructs also included an antibody recognition sequence (the hemagglutinin epitope). Thirty-six hours later, the cells were serum starved in 2% fetal calf serum overnight before the experiments.

C6 glioma cell lines were plated in 100-mm dishes in DMEM plus 10% fetal calf serum 36 h before the experiments. Twelve hours before the experiments, cells were starved in DMEM plus 2% fetal calf serum.

Experimental Treatment and Immunoprecipitation

Immediately before experiments, serum-containing medium was removed and cells were washed once with serum-free medium. Opioid agonists and antagonists were then applied for various time periods, and then cells were rinsed once in phosphate-buffered saline and lysed in 1 ml lysis buffer (10 mM NaCl, 20 mM PIPES (*p*H 7.0), 0.5% NP-40, 0.05% 2-mercaptoethanol, 5 mM EDTA, 50 mM NaF_2, 100 μM Na_3VO_4, 1 mM benzamidine, and 10 μg/ml leupeptin). One hundred nanomolar PMA (4-phorbol 12-myristate 13-acetate) was used as a positive control for ERK activation. Cells were spun at 13,000 rpm for 5 min at 4°C, and then the pellet was discarded. Lysates were equalized for total protein (BioRad, Hercules, CA). Precipitation of MAPKs from the lysate was performed for 1 h using 2 μl of either 12CA5 antibody (raised against the hemagglutinin-epitope, sequence YPYDVPDYA; mouse ascites, a gift of Dr. B. Zanke), anti-ERK antibody (Santa Cruz Biotechnology [Santa Cruz, CA]; a 50:50 mix of ERK-1 [SC-93] and ERK-2 [SC-154] selective antibodies), anti-p46βSAPK, or anti-p38 antibody (courtesy of Dr. B. Zanke). The anti-ERK-1 antibody is directed against amino acids 352–367 of the carboxyl terminus of ERK-1 and is minimally cross-reactive with ERK-2 but not other MAPKs, whereas anti-ERK-2 is directed against amino acids 345–358 of the carboxyl terminus and is minimally cross-reactive with ERK-1 but not other MAPKs. The SAPK and p38 antibodies were raised against full-length, bacterially expressed rat SAPKβ and p38α, respectively, with minimal cross-reactivity toward other MAPK homologs. Protein A-sepharose beads were added for 30 min and the resultant complexes were washed four times with phosphate-buffered saline-T (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4, 0.1% Triton X-100, 50 mM NaF_2, 100 μM Na_3VO_4, 1 mM benzamidine, and 10 μg/ml leupeptin).

Kinase Assays

Kinase assays were performed by adding kinase buffer (10 mM $MgCl_2$, 50 mM Tris [*p*H 7.5], 1 mM EGTA [*p*H 7.5], 40 μM ATP, 40 μCi/ml [γ^{32}P]-ATP) was added to the MAPKs complexed to washed beads and incubated

at 30°C for 30 min with the appropriate substrate. The reaction was terminated using 2× sodium dodecyl sulfate sample buffer, and heated to 95°C for 5 min. prior to loading on 15% sodium dodecyl sulfate - polyacrylamide gel electrophoresis gels. For ERK and p38 assays, 2 μg myelin basic protein (Gibco, Grand Island, NY) was used as a substrate. For SAPK, a c-jun 5-89-glutathione-S transferase fusion protein was produced in the pLysS strain of *Escherichia coli* and used as a substrate.[25] Proteins were affinity purified on glutathione agarose beads and eluted with 20 mM reduced glutathione, 50 mM Tris, *p*H 8. Eluted proteins were dialyzed against a buffer containing 25 mM Tris [*p*H 7.4], 5 mM EDTA, 0.1% Triton-X-100, 2 mM DTT, and 50% glycerol. Jun-associated kinase assays were also performed to measure SAPK activation using the c-jun-GST fusion protein immobilized on glutathione-agarose beads as a substrate. Then ^{32}P-ATP was added in kinase buffer (10 mM $MgCl_2$, 50 mM Tris [*p*H 7.5], 1 mM EGTA [*p*H 7.5], 40 μM ATP, 40 μCi/ml [γ^{32}P]-ATP) and incubated at 30°C for 30 min. Autoradiograms were obtained using Kodak (Rochester, NY) XAR film or phosporimager screens, and quantitation was performed using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Immunoblotting

Lysates were prepared and total proteins equalized as described before and resolved by sodium dodecyl sulfate - polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene fluoride membranes, which were blocked with 1% bovine serum albumin in wash solution (0.1% Tween-20, 100 mM NaCl, 10 mM Tris [*p*H 7.4]) for 1 h and incubated with either anti-phospho ERK or anti-phospho MKK3 antibodies (New England Biolabs, Missisaya, ONT) at 37°C for 1 h. Anti-phospho MAPK detects phosphorylation of tyrosine 204 of p44 and p42 ERKs and does not appreciably cross-react with the corresponding phosphorylated tyrosine of either SAPK or p38 MAPK. Anti-phospho MKK3/6 detects phosphorylation of serine 189 of MKK3 and serine 207 of MKK. This antibody is minimally cross-reactive with the corresponding phosphorylated serine of either SEK or MEK. Secondary anti-rabbit antibody conjugated to horseradish peroxidase (Amersham, Arlington Heights, IL) was then added, the membranes were further washed seven times, and developed using enhanced chemiluminescence (Amersham).

Unless otherwise indicated in figure legends, gels presented are representative of at least three experiments. Graphic data are presented as means ± SEM.

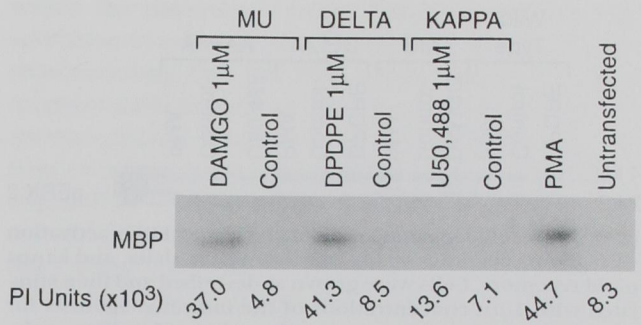


Fig. 2. Extracellular signal-regulated kinase (ERK) activation in transfected COS-7 cells. Cells were grown and transiently transfected with 5 μg hemagglutinin–ERK-2 plasmid and 5 μg opioid receptor indicated above the bracket as described in the text. Cells were subsequently exposed to the indicated doses of opioids or control solution for 30 min and then lysed. Unless otherwise indicated in figure legends, all gels are representative of three experiments. PMA, 4-phorbol 12-myristate 13-acetate; MBP, myelin basic protein PI units, which are arbitrary phosphorimager quantitation units.

Results

Assessment of Extracellular Signal-related Kinase Responses to Opioids in Transient Transfections

Transient introduction of opioid receptors into COS cells was undertaken to determine whether opioids activated MAPK cascades before developing cell lines stably expressing opioid receptors. COS-7 cells were transiently contransfected with opioid receptors and hemagglutinin-tagged ERK-2 using calcium-phosphate precipitation. Stimulation of mu and delta receptors with 1-μM concentrations of the mu agonist DAMGO and the delta agonist DPDPE caused strong activation of ERK-2. However, kappa receptor stimulation with 1 μM of the agonist U50,488 only mildly activated ERK-2 (figure 2). Opioid agonists showed weak (0.5 - 3 times), inconsistent activation of SAPK (data not shown). p38 ERK was not activated by any of the opioid receptor types in this system (data not shown).

Assessment of Extracellular Signal-related Kinase Responses to Opioids in Stable Cell Lines

Once these results were obtained, the effect of opioids on ERK activation in cells stably expressing lower amounts of opioid receptor was undertaken. Immu-

Fig. 3. Extracellular signal-regulated kinase (ERK) activation by C6 glioma cell lines stably expressing mu, delta, and kappa opioid receptors. Cells were grown as described and then stimulated with 1-μM concentrations of the indicated agonists for 30 min. Lysates were run on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels, transferred, and probed with an anti-phospho ERK antibody recognizing the phosphorylated form of both ERK-1 (44 kD) and ERK-2 (42 kD). pERK 2, phosphorylated ERK protein control.

noblotting with an antibody directed against the phosphorylated (activated) forms of ERK revealed strong ERK activation by DAMGO and DPDPE acting at the mu and delta receptors (figure 3). However, U50,488, which acts at kappa receptors, did not activate ERK (figure 3). None of the opioid receptor subtypes activated SAPK or p38 in the stable cell lines (data not shown). To begin to determine the potential physiologic relevance of this activation for opioid-mediated effects such as analgesia, the time course of ERK activation was characterized in mu-receptor-expressing cells. Pilot experiments showed half-maximal ERK activation by DAMGO 2 min after exposure, whereas peak activation was observed 5 min after stimulation (figure 4). Interestingly, the duration of ERK activation was dose dependent. At 1- and 10-nM DAMGO doses, ERK activation levels returned to near baseline by 30 min, whereas at the 100-nM dose (and 1 μM, data not shown), intense initial activation was followed by a sustained lesser (approximately fivefold) activation that persisted for more than 1 h after opioid exposure (figure 4).

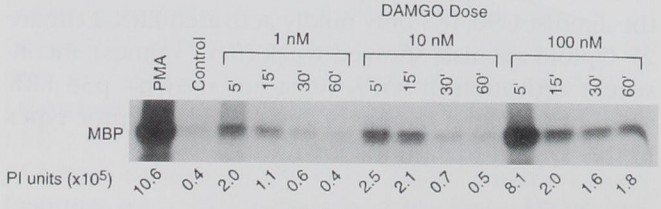


Fig. 4. Time course of extracellular signal-regulated kinase (ERK) activation by D-Ala^2, N-Me-Phe^4, Gly^5-ol enkephalin (DAMGO). Mu receptor–expressing C6 cells were treated with the indicated concentrations of DAMGO for the times noted. PMA (4-phorbol 12-myristate 13-acetate) and control exposures lasted 30 min.

To determine if ERK activation occurred at physiologically relevant concentrations, we identified a dose-response curve for ERK activation using the mu-expressing cell line with fentanyl as an agonist (figure 5A). The median effective concentration for ERK activation by fentanyl was approximately 11 nM (figure 5B), well

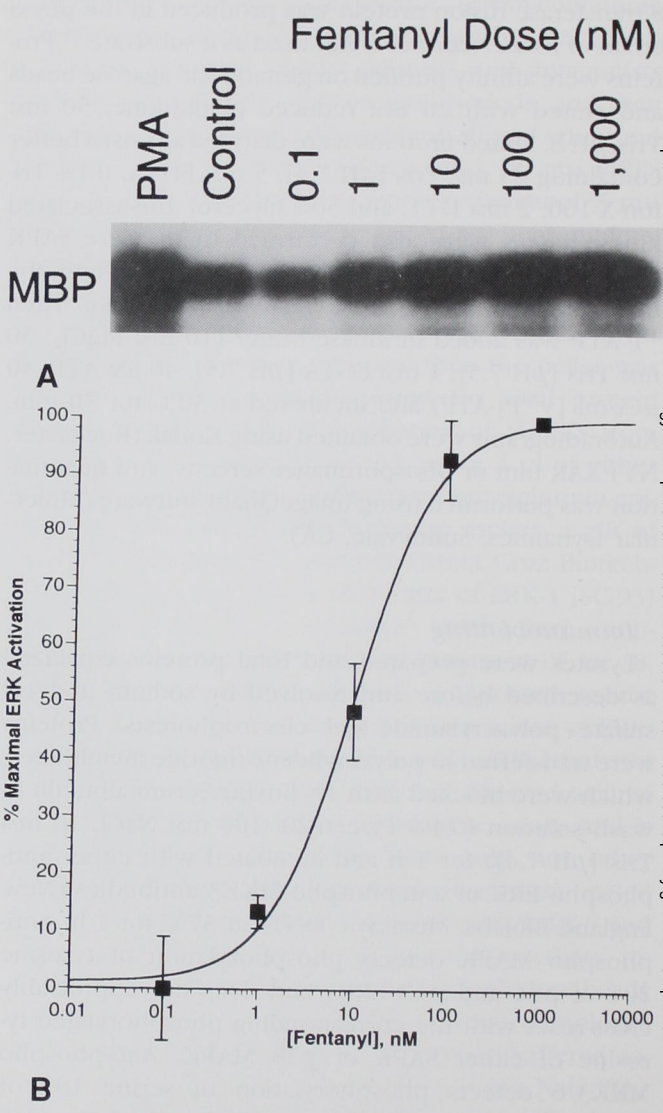
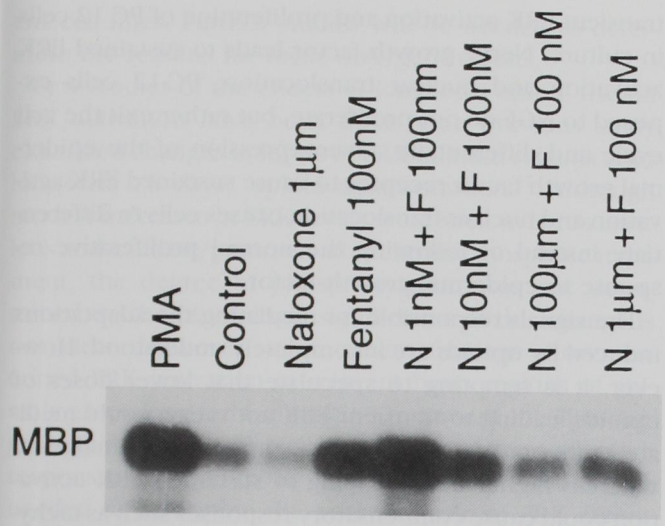


Fig. 5. Dose response of extracellular signal-regulated kinase (ERK) activation by fentanyl. (*A*) Mu receptor–expressing C6 cells were treated with the indicated concentrations of fentanyl or control for 5 min. PMA (4-phorbol 12-myristate 13-acetate) exposure was for 20 min. (*B*) Dose-response curve for ERK activation by fentanyl. Activation is expressed as a percentage of the maximal activation caused by fentanyl in each experiment. Values are means ± SEM for two independent experiments. The equation for the line is y = 99/(1 + (×10)∧−0.9) + 1, R^2 = 0.99.



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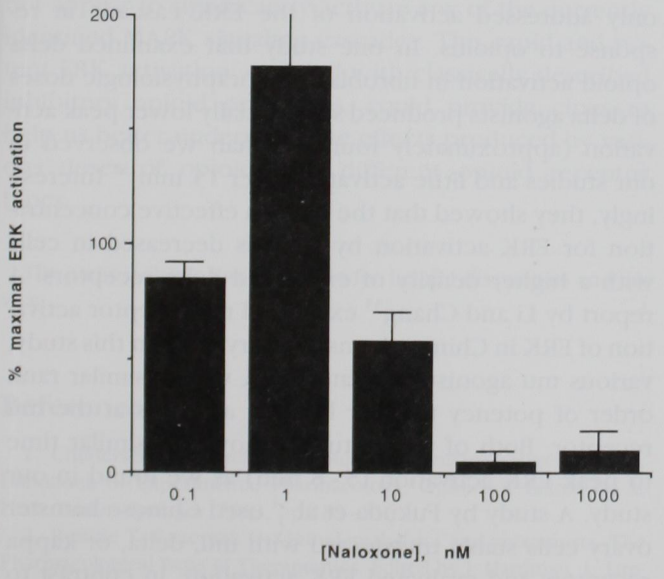

B

Fig. 6. Inhibition of fentanyl-induced extracellular signal-regulated kinase (ERK) activation by naloxone. (*A*) Mu receptor–expressing C6 cells were pretreated with the indicated concentrations of naloxone (or vehicle, for fentanyl treatment alone) for 15 min, and then 100 nM fentanyl was added for 5 min. Activation of ERK above baseline can be seen with 1 nM naloxone pretreatment. (*B*) Dose-response curve for inhibition of ERK activation caused by naloxone. Activation was induced as in panel *A*. Activation is expressed as a percentage of the activation caused by 100 nM fentanyl alone in each experiment. Values are means ± SEM of four independent experiments.

within the physiologic range. Naloxone inhibited this activation in a dose-dependent manner (figures 6A, 6B), demonstrating that the ERK activation was an opioid receptor-mediated effect. The one exception to this response pattern was the 1-nM dose of naloxone. This dose of naloxone increased ERK activation above levels induced by fentanyl in the absence of naloxone. This paradoxical effect occurred when naloxone was applied at the same time as fentanyl, or as early as 15 min before the agonist. It was also observed in pilot studies when DAMGO was used as a mu agonist, and with agonist exposure times as long as 30 min. Naloxone alone, at the 1 nM or any other tested dose, did not activate ERKs (figure 7).

Discussion

We have presented data showing that acute opioid administration, in addition to classically described inhibitory signaling properties, strongly activates the ERK cascade in C6 glioma cells stably expressing opioid receptors. This activation appears to be selective, because the SAPK and p38 cascades were not induced by opioids. Similar activation and selectivity is also seen in transiently transfected COS-7 cells. The slight degree of SAPK activation seen in transfected cells (and mild ERK activation caused by kappa opioids) could be due to promiscuous coupling of overexpressed opioid receptors to effector molecules that would not be used under normal circumstances. The fact that overexpression of

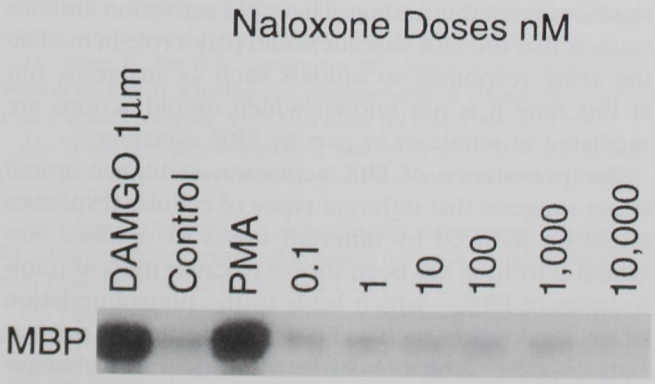

Fig. 7. The effect of naloxone on ERK activation. Mu receptor–expressing C6 cells were exposed to control, 4-phorbol 12-myristate 13-acetate (PMA), and varied concentrations of naloxone for 30 min. DAMGO (D-Ala^2, N-Me-Phe^4, Gly^5-ol enkephalin) exposure lasted 5 min.

receptors can lead to coupling with nonphysiologic messengers is a concern with any *in vitro* cell culture system. In whole rat brain, receptor densities for the three opioid receptor types is between 0.1–0.2 pm/mg protein,[26,27] which is one fifth to one tenth of the levels of expression seen in our cell lines. Some data show that in nucleus accumbens, an area important in mediating opioid responses,[3] the mu receptor density is about 0.4 pm/mg protein,[28] about one fifth that seen in our mu-expressing cell line. However, recent work by Nestler's group indicates that ERK activation by mu opioids occurs in vivo in relevant brain regions,[29] suggesting that our findings may be physiologically relevant.

Extracellular signal-related kinase activation occurred rapidly, peaking 5 min after opioid exposure. At lower agonist concentrations, the activation also resolved quickly, returning to baseline levels within 30 min. Higher mu agonist doses caused a high initial peak of activation, followed by sustained lower-level responses that persisted. Naloxone generally inhibited mu-opioid–induced ERK activation in a dose-dependent manner, indicating that ERK activation was a receptor-mediated phenomenon. The increase in ERK activation that occurred at only the 1-nM naloxone dose was puzzling. Naloxone alone, at various doses and exposure times, did not activate ERK. It is possible that this dose of naloxone could function to sensitize or facilitate agonist-induced opioid signaling. An early study showed a similar enhancement of morphine self-administration in monkeys by low-dose naloxone,[30] and other early work showed a similar paradoxical effect on morphine-induced phosphate incorporation into phospholipids.[31] Future work will attempt to define the mechanism and significance of this finding. The rapid activation kinetics suggest that the ERK cascade could play a role in mediating acute responses to opioids such as analgesia, but at this time it is not known which opioid actions are mediated in whole or in part by ERK signaling.

The persistence of ERK activation at higher opioid doses suggests that different types of cellular responses could be induced by different doses of opioids. Sustained activation has been shown to cause nuclear translocation of ERK,[13] which leads to the phosphorylation of nuclear targets such as Elk-1.[20] Activation of nuclear transcription factors could lead to additional changes in gene expression and unique changes in cellular function. An example of this concept is the difference in ERK activation and cellular responses to nerve growth factor and epidermal growth factor.[13] The latter causes transient ERK activation and proliferation of PC 12 cells in culture. Nerve growth factor leads to sustained ERK activation and nuclear translocation. PC-12 cells exposed to NGF do not proliferate, but rather exit the cell cycle and differentiate. Overexpression of the epidermal growth factor receptor to cause sustained ERK activation and nuclear translocation causes cells to differentiate instead of following the normal proliferative response to epidermal growth factor.[13]

The signals responsible for mediating the adaptations induced by opioids are incompletely understood. However, it is tempting to speculate that lower doses of opioids leading to transient ERK activation could mediate acute opioid effects such as euphoria and analgesia, whereas higher doses leading to sustained ERK activation could cause compensatory responses such as tachyphylaxis or acute tolerance and possibly may relate to longer-term tolerance and physical dependence. Other reports characterizing parts of the acute responses of MAPK cascades to opioids have appeared. These studies only addressed activation of the ERK cascade in response to opioids. In one study that examined delta-opioid activation in fibroblasts, supraphysiologic doses of delta agonists produced substantially lower peak activation (approximately fourfold) than we observed in our studies and little activation after 15 min.[32] Interestingly, they showed that the median effective concentration for ERK activation by opioids decreased in cells with a higher density of expressed delta receptors. A report by Li and Chang[33] examined mu receptor activation of ERK in Chinese hamster ovary cells. In this study various mu agonists activated ERK with a similar rank order of potency to their binding affinities at the mu receptor. Both of these studies showed a similar time to peak ERK activation (5–8 min) as we found in our study. A study by Fukuda et al.[34] used Chinese hamster ovary cells stably transfected with mu, delta, or kappa receptors and evaluated ERK activation. In contrast to our data, stimulation of kappa receptors led to MAPK activation. This could be due to differences in messenger systems available for coupling to kappa receptors in the different cell types. In the present study, we used two different kappa receptor clones (rat and human) and two different types of expression systems (transient and stable transfection) in COS cells and C6 glioma cells, and still we could not demonstrate MAPK activation by kappa stimulation. It is possible that different signaling systems could be engaged by opioid receptors in differ-

ent cell lines. Further studies will be needed to determine the reasons for these divergent results.

Few studies of the effects of long-term opioid use on ERK activation have been done. One previous study examined changes in levels of ERK in brain regions after long-term (6 days) opioid administration.[35] Although modest increases in ERK levels were seen in locus ceruleus and caudate/putamen after chronic morphine treatment, the degree of phosphorylation was not determined, so the physiologic significance of this change is not clear. However, more recent findings show sustained ERK activation in relevant brain regions of animals given long-term morphine treatment.[4,29] This suggests that ERK could play a role in long-term adaptive responses to opioids in vivo. Additional studies are needed to determine the potential role of ERK activation in acute opioid signaling.

In conclusion, we found that mu and delta opioids strongly activate ERK but do not strongly activate either the SAPK or p38 MAPK cascades. Kappa agonists do not appear to significantly activate any of the currently identified MAPK signaling cascades. This rapid and potent ERK activation, coupled with classically described inhibitory opioid properties, could provide clues to help us better understand the effects produced by various doses of opioids and different opioid receptor types.

The authors thank Vuk Stambolic for helpful discussions and advice.

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