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# Dual signal transduction through delta opioid receptors in a transfected human T-cell line

(calcium mobilization/cAMP/Jurkat cells)

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**ABSTRACT** **Opiates are known to function as immunomodulators, in part by effects on T cells. However, the signal transduction pathways mediating the effects of opiates on T cells are largely undefined. To determine whether pathways that regulate free intracellular calcium ($[Ca^{2+}]_i$) and/or cAMP are affected by opiates acting through delta-type opioid receptors (DORs), a cDNA encoding the neuronal DOR was expressed in a stably transfected Jurkat T-cell line. The DOR agonists, deltorphin and [D-Ala$^2$,D-Leu$^5$]-enkephalin (DADLE), elevated $[Ca^{2+}]_i$, measured by flow cytofluorometry using the calcium-sensitive dye, Fluo-3. At concentrations from $10^{-11}$–$10^{-7}$ M, both agonists increased $[Ca^{2+}]_i$ from 60 nM to peak concentrations of 400 nM in a dose-dependent manner within 30 sec ($ED_{50}$ of $\approx 5 \times 10^{-9}$ M). Naltrindole, a selective DOR antagonist, abolished the increase in $[Ca^{2+}]_i$, and pretreatment with pertussis toxin was also effective. To assess the role of extracellular calcium, cells were pretreated with EGTA, which reduced the initial deltorphin-induced elevation of $[Ca^{2+}]_i$ by more than 50% and eliminated the second phase of calcium mobilization. Additionally, the effect of DADLE on forskolin-stimulated cAMP production was determined. DADLE reduced cAMP production by 70% ($IC_{50}$ of $\approx 10^{-11}$ M), and pertussis toxin inhibited the action of DADLE. Thus, the DOR expressed by a transfected Jurkat T-cell line is positively coupled to pathways leading to calcium mobilization and negatively coupled to adenylate cyclase. These studies identify two pertussis toxin-sensitive, G protein-mediated signaling pathways through which DOR agonists regulate the levels of intracellular messengers that modulate T-cell activation.**

Both neuroendocrine peptides and biogenic amines are established immunomodulators. These agents interact with seven transmembrane G protein-coupled receptors that are expressed by mononuclear cells involved in host defense and immunity. Neuropeptides such as substance P (1) and endocrine peptides like adrenocorticotropin (ACTH; secreted by the anterior pituitary gland) modify various immune functions through receptors similar to those found in the central nervous system (2, 3). Norepinephrine, a monoamine secreted from the axon terminals innervating lymphoid tissue (4), stimulates cAMP production through the $\beta_2$-adrenergic receptors present on mononuclear cells. The enkephalins are an example of opioid peptides that most commonly originate from neural tissues, yet are also synthesized by immune cells for the purpose of autocrine/paracrine regulation (5–7). Thus, peptides and biogenic amines secreted from neural, endocrine, and immune tissues directly impact immune function.

Functional and structural studies provide evidence of opioid receptors on lymphocytes. Both enkephalins and synthetic opioid peptide agonists selective for the delta subtype of opioid receptor (DOR) were shown to rapidly stimulate chemotaxis by highly purified human peripheral blood T cells (8). The opioid receptor antagonist, naloxone, blocked these effects and also inhibited the chemotaxis induced by selective mu opioid receptor agonists. In another study, proliferation of highly purified murine splenic T cells was inhibited by DOR agonists (9). Recently, it was reported that simian peripheral blood mononuclear cells express the DOR mRNA originally cloned from hybrid neural cells and present within the central nervous system (10) Expression of the neuronal mu opioid receptor in human peripheral blood CD4$^+$ T cells was also shown by sequencing a partial transcript derived by reverse transcription and polymerase chain reaction (11). Taken together, both pharmacological and molecular approaches indicate that lymphocytes obtained from human, simian, and murine tissues express opioid receptors that are similar to those present in neural tissues. In addition, opioid peptides such as $\beta$-endorphin bind to and modulate lymphocyte function through nonopioid receptors (12–14).

Opiate modulation of both humoral and cell-mediated immunity is due in part to effects on T lymphocytes (15–17). DOR agonists, such as deltorphin, have been shown to modulate crucial events involved in T-cell activation, including (*i*) the proliferative response to crosslinking the CD3 complex associated with the T-cell antigen receptor (TCR) and (*ii*) the production of interleukin 2, which is involved in progression from G1 to S phase of the cycle cycle. At nanomolar concentrations, deltorphin modulated proliferation and interleukin 2 accumulation by both highly purified murine splenic CD4$^+$ and CD8$^+$ T cells (9), and the specific DOR antagonist, naltrindole (18), reversed the effect of deltorphin. Since preincubation with delta opioid agonists was required before stimulation with the anti-CD3 antibody, these studies (9) provide evidence for functional DORs on quiescent splenic CD4$^+$ and CD8$^+$ T-cells.

Opiates are known to affect both free intracellular calcium concentration ($[Ca^{2+}]_i$) and intracellular cAMP concentration (reviewed in ref. 19). Intracellular calcium mobilization is an important early event involved in T-cell activation and proliferation. Crosslinking the TCR is accompanied by a rapid increase in $[Ca^{2+}]_i$ (20–22). The rise in $[Ca^{2+}]_i$ is dependent on calcium release from intracellular stores (23), in response to the generation of inositol 1,4,5-trisphosphate ($IP_3$; ref. 17), and on the influx of extracellular calcium (23). Indeed, the major increment in $[Ca^{2+}]_i$ induced by TCR crosslinking is dependent on extracellular calcium (24).

Intracellular cAMP concentration modulates the activation of phospholipase C (PLC)-$\gamma$1 and the interaction between Ras

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Abbreviations: DOR, delta-type opioid receptor; TCR, T-cell antigen receptor; $[Ca^{2+}]_i$, free intracellular calcium concentration; $IP_3$, inositol 1,4,5-trisphosphate; PLC, phospholipase C; DADLE, D-Ala-D-Leu-enkephalin.

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and Raf, key effectors in pathways that mediate TCR signaling. In lymphocytes, cAMP analogs and forskolin have been shown to retard the generation of $IP_3$ by inhibiting the activity of PLC-γ1 (25–27). Studies in a nonlymphoid cell line identified the Ras-dependent activation of Raf-1 as sensitive to inhibition by increased intracellular cAMP concentration (28). Interleukin 2 production, T-cell cycle progression and T-cell proliferation are also known to be suppressed by increased intracellular cAMP concentration (25, 29). Although the mechanism(s) underlying suppression of interleukin 2 production and T-cell proliferation have not been fully clarified, the inhibitory effects of cAMP on both PLC-γ1 and the Ras–Raf interaction may contribute to impaired signaling through the TCR.

To understand DOR-mediated signal transduction in T-cells and the consequences of this for T-cell function, the cDNA encoding this receptor was stably expressed in Jurkat cells, a human T-cell line. This model was used to determine whether intracellular calcium and cAMP mediate signaling through the neuronal DOR expressed by T-cells. In contrast to studies using splenocytes or even highly purified T-cells, which are both heterogenous with respect to phenotype, function and responsiveness to opioids, this model can advance our understanding of opioid immunomodulation by clarifying the mechanism(s) of DOR-mediated signal transduction in a homogenous population of T-cells.

## MATERIALS AND METHODS

**Reagents.** Deltorphin was obtained from Multiple Peptide Systems, [D-Ala$^2$,D-Leu$^5$]-enkephalin (DADLE) and forskolin were obtained from Sigma, [D-Pen$^2$,D-Pen$^5$]-enkephalin was obtained from Chiron (CMPS), [$^3$H]DADLE and $^{125}$I-labeled cAMP were obtained from New England Nuclear, and Fluo-3 was obtained from Molecular Probes. Cholera and pertussis toxins, and their respective β subunits, were from List Biological Laboratories (Campbell, CA). Naltrindole and antiserum to cAMP (30) were provided by P. Portoghese and T. Walseth (University of Minnesota), respectively, and SNC-80 was obtained from Kenner Rice (National Institutes of Health).

**Transfection and Identification of Jurkat Cells Expressing DOR.** The coding sequence of murine Flag-DOR (obtained from M. von Zastrow, University of California, San Francisco) was cloned into the *Hin*dIII/*Xho*I sites in the REP-9 expression vector (Invitrogen). A Jurkat cell subline, Ju.1, (31) was transfected with murine Flag-DOR pREP-9 by electroporation, and G418 drug-resistant cells were sorted by flow cytometry for Flag-positive cells using M2 anti-Flag mAb (International Biotechnologies; stably transfected DOR-positive cells are designated DOR-Ju.1).

**Measurement of Free Intracellular Ca.** Cells were loaded with Fluo-3 (acetoxy-methyl ester; 8.0 μM) at 37°C for 30 min in the dark, washed once, and resuspended in assay buffer (145 mM NaCl/5 mM KCl/1 mM $CaCl_2$/1 mM $Na_2HPO_4$/0.5 mM $MgSO_4 \cdot 7H_2O$/10 mM Hepes/5 mM glucose, pH 7.4). Cytofluorometric analyses were performed using an EPICS XL flow cytometer (Coulter) equipped with an argon laser, filtered for excitation at 488 nm and emission at 526 nm. Basal cellular fluorescence was measured for 60 sec; thereafter, cell flow was discontinued, specified reagents were added, and then flow resumed at $5 \times 10^4$–$6 \times 10^4$ cells/min. Cells were maintained at 37°C throughout an experiment. Data were displayed and analyzed using MULTITIME (Phoenix Flow Systems, San Diego). The individual points in the graphs depicting the relationships between mean $[Ca^{2+}]_i$ (obtained from mean fluorescence intensity) versus time are not separate data points; rather, they symbolize different lines for continuous measurements over the indicated time periods. Each experiment was performed at least three times, and representative studies are shown in the figures.

**Calculation of $[Ca^{2+}]_i$.** The method of Tsien *et al.* (20) was used with the following equation: $[Ca^{2+}]_i = K_d(F - F_{min})/(F_{max} - F)$, where $K_d$ is 450 nM for Fluo-3 and $F$ is the observed fluorescence level. For each assay, the fluorescence signal of the intracellular Fluo-3 was calibrated. Ionomycin (8 μM)-induced calcium influx was used for measuring $F_{max}$, and EGTA (50 mM) was then added to obtain $F_{min}$.

**Receptor–Ligand Binding Assay.** Intact cells ($5 \times 10^6$) were dispensed into 96-well polypropylene microtiter plates and then incubated for 2 hr at 20°C with [$^3$H]DADLE alone or in combination with unlabeled DADLE or other ligands in 200 μl of Hanks' balanced salt solution, deficient in calcium and magnesium, containing 100 μM phenylmethylsulfonylfluoride and 10 mM $MgCl_2$ (pH 7.4). Saturation binding studies used eight concentrations of [$^3$H]DADLE from 0.1–30.0 nM, each in triplicate. Incubations were terminated by transferring the well contents to Whatman glass fiber filters and then washing three times with 3 ml of ice-cold buffer. Filters were air-dried, placed in liquid scintillation fluid, and counted. Data were analyzed by LIGAND from Biosoft (Cambridge, U.K.).

**cAMP Assay.** DADLE and forskolin were added to $10^5$ Jurkat cells in 0.5 ml of modified Kreb's buffer at 4°C and then incubated for 15 min at 37°C. Total cAMP (incubation medium and cells) was measured by radioimmunoassay, using the general method of Harper and Brooker (32).

## RESULTS

**Expression of DOR by Transfected Ju.1 Cells.** Saturation binding studies showed that [$^3$H]DADLE bound to intact DOR-Ju.1 cells with >90% specificity (unlabeled DADLE $10^{-6}$ M). A single binding site (Hill coefficient = 0.98) with a $K_d$ of 6.5 ± 1.2 nM and 62,700 receptors/cell was identified. Displacement studies with 2 nM [$^3$H]DADLE showed $K_i$ values (mean ± SEM) as follows: 0.26 ± 0.09 nM for naltrindole, 2.88 ± 0.59 nM for deltorphin, 5.56 ± 1.30 nM for SNC-80, 6.40 ± 1.08 nM for DADLE, and 28.9 ± 1.80 nM for [D-Pen$^2$,D-Pen$^5$]enkephalin. Ju.1 cells that had not been transfected with DOR expressed 2800 binding sites/cell.

**Stimulation of Increased $[Ca^{2+}]_i$ by Deltorphin or DADLE.** Within 20–30 sec of adding deltorphin, a selective DOR agonist, to DOR-Ju.1 cells, a rise in mean $[Ca^{2+}]_i$ was evident (Fig. 1; deltorphin added at +60 sec; all calcium concentrations are shown as mean values). Deltorphin maximally elevated $[Ca^{2+}]_i$ within 40–70 sec (peak I); at higher concentrations of deltorphin ($10^{-7}$–$10^{-5}$ M), a secondary elevation of $[Ca^{2+}]_i$ was evident at ≈150 sec and thereafter. Following this, $[Ca^{2+}]_i$ levels declined gradually, but remained significantly above basal levels for more than 600 sec.

As shown in Fig. 2, the mobilization of intracellular calcium by deltorphin or DADLE was dose-dependent (approximate $ED_{50}$s of $5 \times 10^{-9}$ M). At $10^{-7}$ M, both deltorphin and DADLE stimulated maximal increases in $[Ca^{2+}]_i$; these were at least five-fold, with $[Ca^{2+}]_i$ rising from 50–80 nM to 400 nM. In contrast to stimulating calcium mobilization by DOR-Ju.1 cells, deltorphin failed to do so in nontransfected Ju.1 cells. The specific DOR antagonist, naltrindole (18), inhibited deltorphin-induced calcium mobilization (Fig. 3). Naltrindole ($10^{-9}$ M) reduced the peak I response to deltorphin $10^{-7}$ M by 70%, abolished the secondary response, and eliminated the gradual decline in $[Ca^{2+}]_i$ levels. Naltrindole ($10^{-8}$ M) abolished the entire calcium response to deltorphin.

**Pertussis Toxin Blocks Deltorphin-Stimulated $[Ca^{2+}]_i$ Mobilization.** The DOR is a G protein-coupled receptor (33) that commonly interacts with the $G_i$ class of G proteins, which inhibit adenylyl cyclase. Many $G_i$-coupled receptors activate isoenzymes of PLC (34–36), and the subsequent elevation of $IP_3$ stimulates an increase in $[Ca^{2+}]_i$. Since signaling through $G_i$-coupled receptors is inhibited by pertussis toxin, which uncouples $G_i$ proteins from their receptors, the effect of

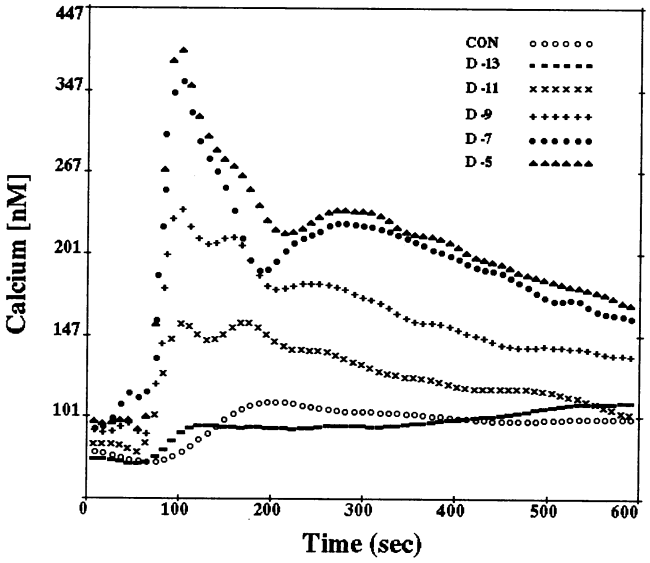


FIG. 1. Dose dependency and time course of the intracellular calcium response to deltorphin in DOR-Ju.1 cells. Cells were loaded with Fluo-3 and then basal fluorescence was measured for 60 sec by flow cytofluorometry. Deltorphin [D; concentrations ($10^{-x}$ M) are shown as −13 to −5] or vehicle control (CON) was injected at +60 sec. Data were displayed and analyzed using MULTITIME; mean values for $[Ca^{2+}]_i$ versus time are shown. The method of Tsien *et al.* (20) was used to calculate $[Ca^{2+}]_i$. Representative experiments are shown (performed at least three times).

pertussis toxin on deltorphin-stimulated calcium mobilization in DOR-Ju.1 cells was studied.

Cells were treated for 6 hr with pertussis toxin (10 or 100 ng/ml) and then stimulated with deltorphin $10^{-7}$ M. Fig. 4 shows that both concentrations of pertussis toxin inhibited 90% of the deltorphin-induced increase in peak I $[Ca^{2+}]_i$ and produced a similar reduction in the $[Ca^{2+}]_i$ levels that follow peak I. In contrast, the $\beta$ subunit of pertussis toxin (membrane binding subunit), at concentrations as high as 1 $\mu$g/ml, was ineffective, and intact pertussis toxin did not reduce ionomycin-induced calcium mobilization (data not shown). Therefore, the inhibitory effect of intact pertussis toxin on deltorphin-induced calcium mobilization was specific.

**EGTA Attenuates Deltorphin-Stimulated $[Ca^{2+}]_i$ Mobilization.** In lymphocytes, $IP_3$-dependent calcium mobilization may involve both the release of calcium from intracellular stores and the influx of extracellular calcium (22, 37). To determine the relative contribution of these two calcium pools to the increase in $[Ca^{2+}]_i$ stimulated by deltorphin, DOR-Ju.1 cells were incubated with the calcium chelating agent, EGTA (10 or 50 mM, final concentration), 2 min before stimulation with deltorphin $10^{-7}$ M. Fig. 5 shows that EGTA reduced the maximum elevation of peak I $[Ca^{2+}]_i$ by 36–58% for 10 versus 50 mM EGTA, respectively. The secondary calcium response, followed by a gradual decline in $[Ca^{2+}]_i$ levels, was eliminated. Therefore, a modest elevation of peak I $[Ca^{2+}]_i$ persists in the presence of EGTA, indicating that deltorphin initially releases calcium from intracellular stores and then from extracellular pools. The secondary calcium response was completely blocked by EGTA, indicating its dependence on the influx of extracellular calcium.

**DADLE Inhibits Forskolin-Stimulated cAMP Production.** In preliminary experiments, the dose-dependent effects of forskolin on cAMP production by DOR-Ju.1 cells was determined. Cells stimulated with forskolin ($10^{-7}$–$5 \times 10^{-5}$ M) for 15 min showed the following cAMP levels (mean ± SEM, $n$ = 3; pmol/$5 \times 10^5$ cells): 1.23 ± 0.05 for basal, 2.77 ± 0.03 at $10^{-7}$ M, 17.41 ± 1.6 at $10^{-5}$ M, and 90.85 ± 5.16 at $5 \times 10^{-5}$ M. Fig. 6 shows that DADLE reduced the cAMP response to a

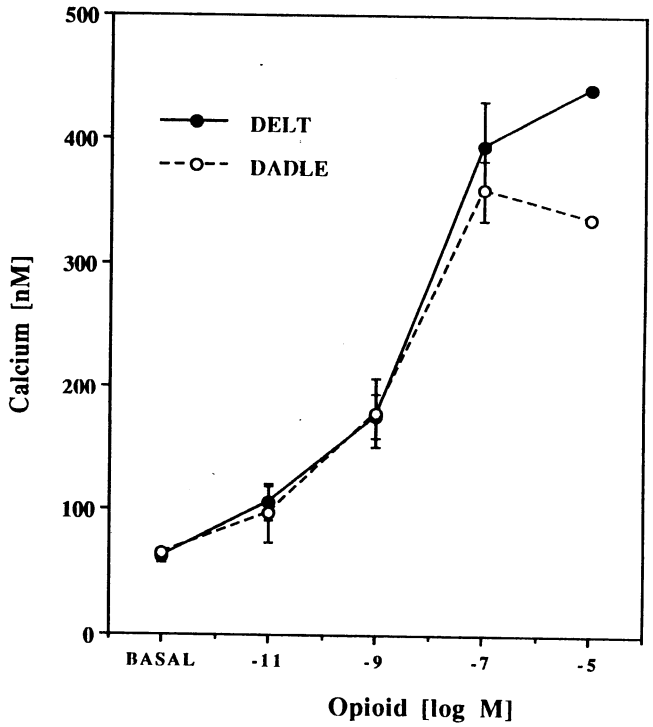


FIG. 2. Maximum $[Ca^{2+}]_i$s in response to deltorphin or DADLE in DOR-Ju.1 cells. Cells were treated as described in Fig. 1. The values (mean ± SEM) for maximal $[Ca^{2+}]_i$ in response to each concentration of opiate agonist were calculated using the highest mean $[Ca^{2+}]_i$ attained during the peak I response from each of three experiments. Both DOR agonists significantly increased $[Ca^{2+}]_i$ at $10^{-9}$ M ($P <$ 0.01) and higher concentrations ($P < 0.001$ for $10^{-7}$ M; $n$ = 1 at $10^{-5}$ M) compared with basal levels. $ED_{50}$ values were $\approx 5 \times 10^{-9}$ M for both agonists.

submaximally stimulative concentration of forskolin ($5 \times 10^{-5}$ M) by 70%. The maximal reduction was achieved by DADLE $10^{-9}$ M (approximate $IC_{50}$ of $10^{-11}$ M).

**Pertussis Toxin Blocks the Effects of DADLE on cAMP Production.** Cells were treated with pertussis toxin (10 or 100

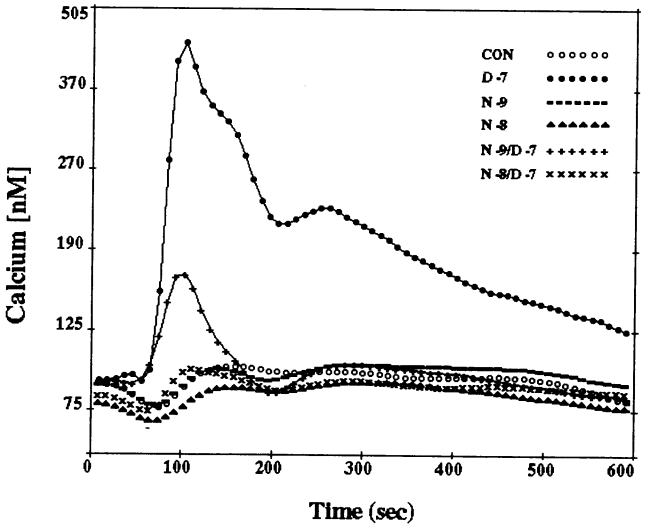


FIG. 3. The DOR antagonist, naltrindole, blocks the intracellular calcium response to deltorphin. DOR-Ju.1 cells were loaded with Fluo-3, naltrindole [N; concentrations ($10^{-x}$ M) are shown as −9 or −8] or vehicle was added 2 min before obtaining basal fluorescence measurements and then deltorphin (D; $10^{-7}$ M) or vehicle was added at +60 sec. Controls (CON) received vehicle twice. Mean values for $[Ca^{2+}]_i$ versus time are shown.

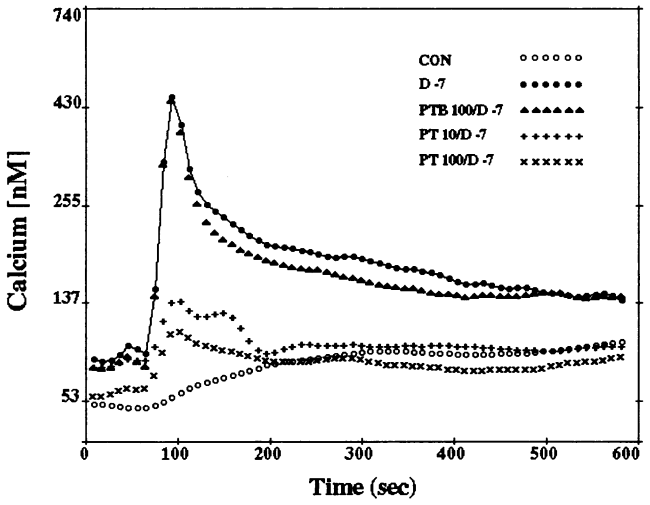


FIG. 4. Pertussis toxin inhibits deltorphin-induced calcium mobilization. DOR-Ju.1 cells were incubated with pertussis toxin (PT; 10 or 100 ng/ml), the $\beta$ subunit of pertussis toxin (PTB; 100 ng/ml) or vehicle for 6 hr before loading with Fluo-3. Then, basal fluorescence was determined and deltorphin (D; $10^{-7}$ M) or vehicle was added at +60 sec. Controls (CON) received vehicle twice. Mean values for $[Ca^{2+}]_i$ versus time are shown. PT had no effect on ionomycin-induced calcium mobilization.

ng/ml) or the $\beta$ subunit of pertussis toxin for 6 hr and then stimulated with forskolin ± DADLE $10^{-11}$ or $10^{-9}$ M ($n$ = 3/treatment). These concentrations of peptide reduced cAMP production to 65.0 ± 6.8% ($P < 0.05$) and 33.0 ± 1.2% ($P < 0.01$) of control (forskolin alone), respectively. The corresponding values obtained after pretreatment with pertussis toxin were 116.0 ± 8.5% and 101.0 ± 6.2% of control. The $\beta$ subunit of pertussis toxin was ineffective (63.3 ± 3.0% and 34.7 ± 3.0% of control).

## DISCUSSION

These experiments have shown that the neuronal DOR expressed by a transfected human T-cell line is coupled to signal

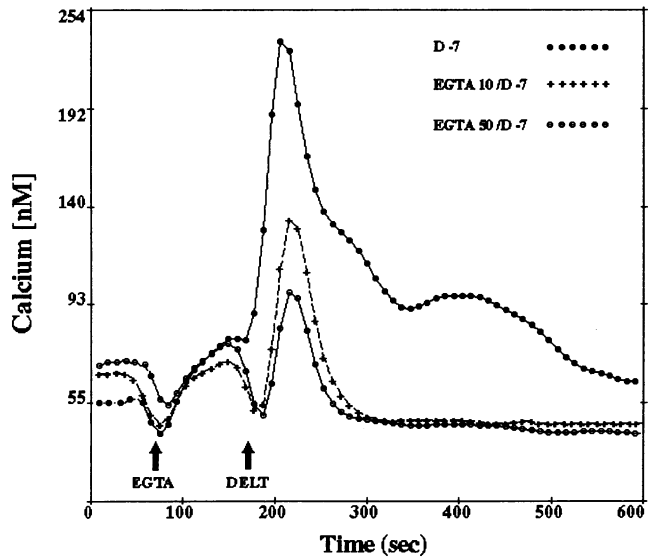


FIG. 5. EGTA inhibits deltorphin-induced calcium mobilization in DOR-Ju.1 cells. Cells were loaded with Fluo-3 and basal fluorescence was measured. EGTA 10 or 50 mM or vehicle was added at +60 sec and deltorphin (D; $10^{-7}$ M or DELT) was added at +180 sec. Mean values for $[Ca^{2+}]_i$ versus time are shown.

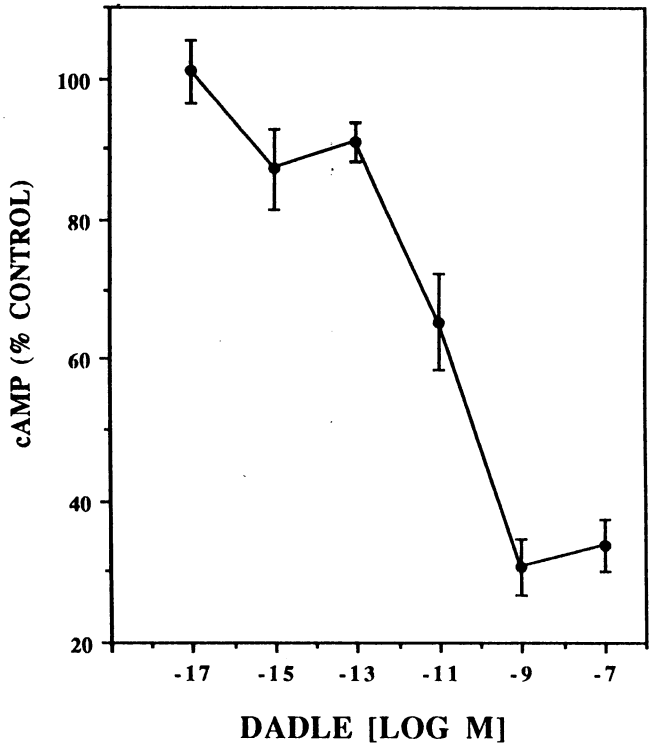


FIG. 6. DADLE inhibits forskolin-stimulated cAMP production by DOR-Ju.1 cells. Cells were cooled to 4°C just before treatment with forskolin ($5 \times 10^{-5}$ M) and DADLE, and then incubated for 15 min at 37°C. Total cAMP (incubation medium and cells) was measured by radioimmunoassay. Data (mean ± SEM) are expressed as percentage of the response to forskolin alone (percent control; forskolin-stimulated cells = 25.9 ± 4.9 pmol/$10^5$ cells and unstimulated cells = 2.3 ± 0.05). DADLE significantly reduced the cAMP response to forskolin ($P < 0.01$ for DADLE $10^{-11}$–$10^{-7}$ M; $n$ = 9 per concentration, based on three experiments).

transduction pathways that regulate intracellular concentrations of calcium and cAMP. Both pathways were sensitive to pertussis toxin; DOR was positively coupled to calcium mobilization and negatively to cAMP synthesis. DADLE inhibited forskolin-stimulated cAMP synthesis at $IC_{50}$ concentrations ≈15-fold lower (10 pM) than those required to elevate $[Ca^{2+}]_i$ ($5 \times 10^{-9}$ M). This increase in $[Ca^{2+}]_i$ reflected the mobilization of calcium from intracellular pools and the influx of extracellular calcium. The influx of extracellular calcium predominated, contributing to the peak I elevation of $[Ca^{2+}]_i$ and accounting for all of the secondary rise and gradual decline, thereafter, in $[Ca^{2+}]_i$. These investigations with transfected T cells indicate that the neuronal DOR expressed by normal T cells can potentially couple to two signal transduction pathways. Both of these pathways are known to affect the T-cell activation program whereby T-cells move from $G_o$ into the cell cycle to undergo clonal expansion and differentiation into specific immune effector cells (20–22, 25, 26, 29).

The elevation of $[Ca^{2+}]_i$ due to stimulation of the T-cell antigen receptor (TCR) is dependent on a rapid release of calcium from intracellular sources followed by the influx of extracellular calcium (23, 24). The initial kinetics of the deltorphin-induced increase in $[Ca^{2+}]_i$ are similar, and both depend predominantly on extracellular calcium. However, following TCR stimulation, $[Ca^{2+}]_i$ is elevated for at least several hours (reviewed in ref. 38), whereas after deltorphin the elevation persists for 15–30 min (data not shown). Since it is thought that increased $[Ca^{2+}]_i$ must be sustained for at least several hours to effectively induce cell proliferation (38), it is unlikely that the elevation of $[Ca^{2+}]_i$ through DOR is sufficient to do so. Nevertheless, the DOR-induced elevation of $[Ca^{2+}]_i$

may interact with second messenger pathways that are activated by the TCR, modulating the production and/or action of cell cycle regulators, such as interleukin 2. This is supported by the observation that deltorphin modulated the secretion of interleukin 2 by highly purified normal splenic $CD4^+$ T-cells that were stimulated through the TCR (9).

Following TCR activation, the initial mobilization of intracellular calcium is stimulated by the $IP_3$ generated by PLC-γ1 (20). The subsequent influx of extracellular calcium may also involve this pathway, which is independent of G proteins (37, 39, 40). In contrast, both phases of the calcium response to deltorphin are pertussis toxin-sensitive, indicating the involvement of the $G_i$ or $G_o$ families of G proteins. The βγ subunits associated with these G proteins are known to activate PLC-β isozymes, such as PLC-$\beta_1$, which has been identified in Jurkat cells, resulting in the synthesis of $IP_3$ (41–43). Therefore, the efficacy of pertussis toxin at blocking calcium mobilization by deltorphin implicates DOR coupling to $G_i$ or $G_0$ proteins and suggests that the subsequent activation of PLC-β isozymes regulates $IP_3$ production in DOR-Ju.1 cells. The absence of a prolonged elevation of $[Ca^{2+}]_i$ and the dependence on G-proteins distingushes the mechanism of DOR-induced calcium mobilization from that mediated by the TCR through the phosphorylation-dependent activation of PLC-γ1 (43).

Numerous studies have shown that opiate agonists modulate calcium channels. In neuroblastoma NG108-15 cells, which express only the delta subtype of opioid receptor, DOR agonists inhibited voltage-dependent calcium channels through a pertussis toxin-sensitive mechanism (19, 44). However, in forskolin-differentiated NG108-15 cells, DOR agonists such as DADLE were reported to increase calcium influx through voltage-gated channels (45). DOR agonists also increased $[Ca^{2+}]_i$ in undifferentiated NG108-15 cells, apparently by mobilizing calcium from intracellular stores (45). Kappa opioid receptor agonists have also been reported to decrease calcium conductance in neuronal preparations (46, 47). In contrast, kappa, mu, and delta agonists increased intracellular $[Ca^{2+}]_i$ in human B lymphocyte lines (ref. 48; all effects were blocked by naloxone), and kappa opioid receptor agonists had similar effects on murine neonatal type 1 astroglial cells (49) and human trophoblastic cells (50). Tissue differences in the specific subunit composition of the G proteins (51) that are coupled to opioid receptors and in the expression of more distal effector mechanisms probably account for the fact that an opioid receptor subtype can have opposing effects on $[Ca^{2+}]_i$.

Whether or not they were treated with forskolin, NG108–15 cells have shown a depolarization-evoked increase in $[Ca^{2+}]_i$ that was inhibited by DOR agonists (44, 45), apparently without affecting $IP_3$ generation (52). Moreover, the inhibition of calcium currents in dorsal root ganglion neurons by the kappa opioid agonist, dynorphin A, was shown to depend on a change in the voltage-dependence with which calcium channels are opened (53). The present study suggests that the elevation of $[Ca^{2+}]_i$ by DOR agonists in DOR-Ju.1 cells depends on a different, albeit pertussis toxin-sensitive, effector mechanism. In these cells, the increase in $[Ca^{2+}]_i$ that was due in part to release from intracellular stores resembles the DADLE-induced increase in $[Ca^{2+}]_i$ observed with undifferentiated NG108–15 cells (45). In this respect, DOR may be coupled to common effector mechanisms in these immune and neural cell lines.

It is well established that activation of DOR results in the inhibition of adenylate cyclase activity in many cell types. This is characteristic of the DOR expressed by normal neural tissue and that expressed by hybrid neural cells (NG108-15) or transfected COS cells (19, 33). The present study indicates that the DOR expressed by a transfected T cell regulates cAMP levels through a similar pertussis toxin-sensitive effector mechanism. However, the $IC_{50}$ for deltorphin in this system was lower than usually observed. This $IC_{50}$ indicates that <10% receptor occupancy was required to inhibit 50% of the forskolin-stimulated cAMP response. Such efficient signal transduction is characteristic of other lymphocyte responses, including those mediated by cytokine (31) and chemokine receptors; the latter are seven transmembrane G protein-coupled receptors (54). For example, RANTES, a chemoattractant peptide, stimulated pertussis toxin-sensitive chemotaxis by T cells with an $ED_{50}$ of $\approx 10^{-11}$ M (55). Although the second messenger(s) mediating its chemotactic effects are not defined, RANTES also increased $[Ca^{2+}]_i$, and the first phase of this response was sensitive to pertussis toxin. Thus, intracellular events regulating highly efficient signal transduction may be similar for lymphocyte DOR and chemoattractant receptors. These events include the specific subunits that constitute the heterotrimeric G proteins mediating signal transduction and regulation of their rates of association/dissociation (56), which may determine the efficacy of coupling to distal effector mechanisms.

These investigations with transfected T cells show that DOR is coupled to two signal transduction pathways that are known to affect T-cell activation. Both the inhibition of adenylate cyclase and the elevation of $[Ca^{2+}]_i$ by an $IP_3$-dependent mechanism have been reported for other pertussis toxin-sensitive, G protein-coupled receptors (57). Therefore, DOR agonists may modulate normal T-cell activation through the coupling of DOR to the signal transduction pathways shown herein.

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