**Delta** Opioid Receptor Subtypes Activate Inositol-Signaling Pathways in the Production of Antinociception

PILAR SÁNCHEZ-BLÁZQUEZ and JAVIER GARZÓN

Neurofarmacología, Instituto de Neurobiología Santiago Ramón y Cajal, Consejo Superior de Investigaciones Científicas, Avenida Doctor Arce 37, E-28002 Madrid, Spain

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**ABSTRACT**

To analyze the selectivity of *delta* receptor subtypes to regulate different classes of G proteins, the expression of the *α*-subunits of G2, G3, G01, G02, Gq and G11 transducer proteins was reduced by administration of oligodeoxynucleotides (ODNs) complementary to sequences in their respective mRNAs. Mice receiving antisense ODNs to G02α, G3α, Gqα and G11α subunits showed an impaired antinociceptive response to all the *delta* agonists evaluated. An ODN to G01α specifically blocked the antinociceptive effect of the agonist of *delta*-1 receptors, [D-Pen²⁵]enkephalin (DPDPE), without altering the activity of [D-Ala²]deltorphin II or [D-Ser²]-Leu-enkephalin-Thr (DSLET). In mice treated with an ODN to G01α, the effects of the agonists of *delta*-2-opioid receptors were reduced, but not those of DPDPE. Thus, G01 proteins are selectively linked to *delta*-1-mediated analgesia, and Gq proteins are related to *delta*-2-evoked antinociception. After impairing the synthesis of G01α subunits, DPDPE exhibited an antagonistic activity on the antinociception produced by [D-Ala²]deltorphin II. After treatment with ODNs complementary to sequences in G01α or PLC-β1 mRNAs, the antinociceptive capacity of [D-Ala²]deltorphin II was diminished. However, the *delta*-2-agonist did not alter the antinociceptive activity of DPDPE. An ODN complementary to nucleotides 7 to 26 of the murine *delta* receptor reduced the analgesic potency of [D-Ala²]deltorphin II, but not that observed for DPDPE. In these mice, [D-Ala²]deltorphin II did not antagonize the effect of DPDPE. These results suggest the existence of different molecular forms of the *delta* opioid receptor, and the involvement of inositol-signaling pathways in the supraspinal antinociceptive effects of *delta* agonists.

A series of studies on the antinociceptive effects of *delta*-selective agonists in the mouse has led to the proposed division of *delta* opioid receptors into subtypes, termed *delta*-1 and *delta*-2 (see for review Zaki et al., 1996). Most of the pharmacological evidence favoring the subdivision of *delta* receptors has come from the use of antagonists (Sofuoglu et al., 1991a; Portoghese et al., 1992; Vanderah et al., 1994). The *delta*-1 receptor was defined as the site activated by DPDPE and sensitive to antagonism by [D-Ala²,Leu³,Cys⁸]-enkephalin and 7-benzylidenenaltrexone, the *delta*-2 receptor binds the agonists [D-Ala²]deltorphin II and DSLET and the antagonist naltrindole 5′-isothiocyanate. Other pharmacological approaches, including tolerance and cross-tolerance studies (Mattia et al., 1991; Sofuoglu et al., 1991b), have also supported the existence of subtypes for the *delta* receptor. Consistent with this proposal, the CXBK inbred strain of mice exhibits a diminished response to i.c.v., but not intrathecal, [D-Ala²]deltorphin II (Raffa et al., 1992). It is proposed that these mice are deficient in the *delta*-2 subtype of the *delta* receptor in the brain, but not in the spinal cord. By contrast, a single functional subtype of *delta* opioid receptor has been described in in vitro bioassay systems, such as the mouse vas deferens (Wild et al., 1993).

The primary structure of a unique *delta* receptor from the adult mouse brain has been obtained by molecular cloning (Yasuda et al., 1994). The mRNA encodes a protein of 372 amino acids that presents a structural homology to G protein-coupled receptors. The ODN strategy has been used to impair *delta* receptor-mediated functions in in vivo studies (Standifer et al., 1994; Lai et al., 1994; Bilsky et al., 1996; Sánchez-Blázquez et al., 1997). After subchronically giving doses of an ODN complementary to sequences in *delta* opioid receptor, the i.c.v. injection of ¹²⁵I-IgGs directed to *delta* receptors demonstrated a reduction of the protein encoded by the targeted mRNA (Sánchez-Blázquez et al., 1997). The in vivo administration of an ODN complementary to nucleotides 7 to 29 of the cloned *delta* receptor did not reduce the antinociceptive potency of all the agonists binding this *delta* receptor.

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**ABBREVIATIONS:** *delta*-1 and *delta*-2, subtypes of the *delta* opioid receptor; DPDPE, [D-Pen²⁵]enkephalin; DSLET, [D-Ser²]-Leu-enkephalin-Thr; G proteins, GTP-binding proteins; PLC, phosphoinositide-specific phospholipase C; ODN, antisense oligodeoxynucleotide; RD, random oligodeoxynucleotide; i.c.v., intracerebroventricular; PAG, periaqueductal gray matter; MPE, maximum possible effect.
It is assumed that, at the cellular level, the effects of opioids involve the inhibition of cAMP formation, the inhibition of Ca\(^{2+}\) entry through voltage-sensitive Ca\(^{2+}\) channels, and the stimulation of an outward K\(^{+}\) conductance (for review, see Childers, 1991). In addition, recent reports suggest the coupling of opioid receptors to the phosphoinositide cascade (Miyamae et al., 1993; Smart et al., 1994; Jin et al., 1994). Thus, it is of interest to determine whether the stimulatory effect of opioids on inositol lipid turnover also participates in the mechanisms of opioid-induced antinociception. It is well established that delta agonists regulate G proteins to produce supraspinal antinociception; this has been ascertained by the *in vivo* administration of agents known to interfere directly with the function of G proteins, i.e., pertussis toxin (Sánchez-Blázquez and Garzón, 1988, 1991), antibodies administered by the i.c.v. route against G\(_{i\alpha}\) and G\(_{i\alpha}\) subunits (Sánchez-Blázquez and Garzón, 1993; Sánchez-Blázquez et al., 1993, 1995), and *in vivo* antisense strategies to reduce the synthesis of specific Ga subunits (Raffa et al., 1994; Sánchez-Blázquez et al., 1995; Standifer et al., 1996).

In our work, we have extended our previous studies on the regulation of G proteins by delta receptors in the production of supraspinal analgesia to include G protein classes associated with inositol lipid turnover: Go1, Go2, Gq and G11. The participation of PLC\(_{\gamma}\) in this opioid activity was also explored. Distinct binding sites and inositol-signaling pathways mediated the supraspinal antinociceptive effects of delta agonists; one was linked to a PTX-sensitive Go1 protein (delta-1), and the other to a PTX-resistant Gq protein (delta-2). These results provide further support for the existence of subtypes of delta receptors.

**Methods**

**Animals and evaluation of analgesia.** Male albino mice CD-1 (Charles River, Barcelona, Spain) weighing 22 to 25 g, were used throughout this study. Animals were kept at 22°C under a 12-h light/dark cycle (8 A.M./8 P.M.). Food and water were provided *ad libitum*. Mice were housed and used strictly in accordance with the guidelines of the European Community about Care and Use of Laboratory Animals. To reduce the possibility of interference from spinal events, all substances were injected i.c.v. into the right lateral ventricle, as previously described (Sánchez-Blázquez et al., 1995, 1997).

Briefly, animals were lightly anesthetized with ether, and injections were given with a 10-μl Hamilton syringe at a depth of 3 mm, 2 mm lateral and 2 mm caudal from the bregma. Four μl of each solution were infused at a rate of 1 μl every 5 sec; afterward, the needle was maintained for an additional period of 10 sec. The warm water (52°C) tail-flick test was used to measure the antinociceptive effect. Latencies in seconds were determined both before treatment (basal latency) and also after the administration of the substance under study. Baseline latencies ranged from 1.5 to 2.2 sec and were not affected by ODN administration. A cut-off time of 10 sec was allotted to minimize the risk of tissue damage. Antinociception was expressed as a percentage of the MPE according to the following formula: %MPE = 100 x (test latency-baseline latency)/cut-off time-baseline latency). Dose-response curves were routinely constructed for the opioids under control and treatment conditions; however, for the sake of directness the data are presented in a single dose format. Intracerebroventricular doses of the opioids were given and antinociception was assessed after 90 min for β-endorphin, after 15 min for DPPE or DSLET and after 10 min for [d-Ala\(_{2}\)deltorphin II. All compounds were dissolved in distilled water except for [d-Ala\(_{2}\)deltorphin II, which was dissolved in 1% DMSO. Agonist solutions were made up immediately before use. Statistical analysis of the results was accomplished by analysis of variance followed by the Student-Newman-Keuls test. The level of significance was set at P < .05.

**Synthesis of oligodeoxynucleotides.** End-capped phosphorothioate ODNs were synthesized in a CODER 300 DNA synthesizer using phosphoramidite chemistry (Matteucci and Caruthers, 1981). Crude ODNs were purified by reverse-phase chromatography using COP cartridges (Cruachem, Glasgow, UK). The eluted ODNs in 50% acetonitrile-water were then lyophilized (Rouan RC 1090/RCT 90, France). Sequences were as follows: ODN-G\(_{i10}\)a: 5‘-T’GGTCACCCAGGCCTCGCTAGCGGAGCGGAG-3‘, corresponding to nucleotides 529-556 of the G\(_{i\alpha}\) gene sequence (Jones and Reed, 1987). This ODN was also labeled in the final synthetic cycle with fluorescein-CE phosphoramidite at the 5‘-end (Cruachem, Glasgow, UK, #22-8409); ODN-G\(_{i10}\)b: 5‘-G’CATCTGCCCCATATAAGTTTTAAATCACGCCT*G’C-3‘, corresponding to nucleotides 554-587 of the G\(_{i\alpha}\) gene sequence (Jones and Reed, 1987). Both oligos were previously characterized (Raffa et al., 1994; Sánchez-Blázquez et al., 1995). ODN-G\(_{i10}\)c: 5‘-A’GGCCATCTTCATCTCTAGGGT’T’C-3‘, a 25 base oligo corresponding to nucleotides 882-906 of the murine G\(_{i\alpha}\) gene sequence; and ODN-G\(_{i10}\)d: 5‘-G’CAGGCGTCTTCGGA**C’G’C-3‘, corresponding to nucleotides 724-744 of the G\(_{i\alpha}\)G\(_{11}\)a gene sequence (Strathmann and Simon, 1990). These two ODNs were identical to those used by Kleuss et al. (1991) in *in vitro* experiments. ODN-G\(_{i10}\)e: 5‘-C’GCTACAGGTGCTACGCTAAG**A’T’G-3‘, corresponding to nucleotides 484-504 of the G\(_{i}\) gene sequence; ODN-G\(_{i10}\)f: 5‘-C’GTGGCGATGGCGCCAC**G’C’G-3‘, which corresponds to nucleotides 487-507 of the G\(_{11}\)a sequence; and ODN-G\(_{i10}\)g: 5‘-**G’C’ATGCCGCTATCTTCTG**T’G’C-3‘, a 21 base oligo corresponding to nucleotides 724-744 of the G\(_{i}\)G\(_{11}\)a gene sequence (Strathmann and Simon, 1990). ODN-delta-7: 5‘-**G’C’ACCGGCAGAGGGCCAC**A’G’C-3‘, corresponding to nucleotides 7 to 26 of the murine delta opioid receptor gene. This ODN is identical to that designed by Lai et al. (1994). And finally, ODN-PLC-β5: 5‘-**G’C’TGCTGCAGACGAC**A’C’-3‘, corresponding to nucleotides 49-63 of the PLC-β1 gene sequence (Suh et al., 1988). These sequences displayed no homology to other relevant cloned proteins (GeneBank database). A random oligo (ODN-RD) with the sequence 5‘-C’C’CTTTATTTACTCTTTGC’G’C’C-3‘ served as a control (Sánchez-Blázquez et al., 1995).

**Administration of ODNs.** ODN solutions were made up in the appropriate volume of sterile water immediately before use. Animals received either the vehicle, the random sequence ODN or the antisense oligo. These were injected into the right lateral ventricle and subsequent administrations were performed on the same side. Each ODN treatment was performed on a distinct group of 15 to 20 mice using the following schedule: on days 1 and 2, with 2 nmol; on days 3 and 4, with 3 nmol; on day 5, with 4 nmol. On day 6, the opioid agonists were injected i.c.v. and their antinociceptive activity evaluated by the warm water tail-flick test. An interval of 24 hr was selected between ODN administrations to minimize neurotoxic damage (Chiaison et al., 1994). With this schedule, the experimental animals displayed no noticeable behavioral changes.

**Visualization at the PAG level of a fluorescence-labeled ODN to G\(_{i10}\)a mRNA.** Mice that had received a single i.c.v. injection of 3 nmol of a fluorescein-labeled ODN-G\(_{i10}\)a were killed 10, 30 or 120 min later. Brains were removed and frozen on dry ice. Coronal cryostat sections (20 μm) were cut, mounted onto gelatin-subbed slides and covered in a solution of 0.1 M phosphate buffer-30% glycerol. Sections were analyzed under a Leica TCS 4D confocal laser-scanning microscope equipped with an argon/krypton-mixed gas laser with excitation peak at 488 nm. The confocal microscope was associated with a Leitz DMIRB fluorescence microscope.
Electrophoresis and immunoblotting. Membranes from PAGs of ODN-treated mice were solubilized in a buffer containing 50 mM Tris.HCl, 3% sodium dodecyl sulfate, 10% glycerol, 5% β-mercaptoethanol, pH 6.8, and the proteins (30 to 60 μg protein/lane) were subsequently resolved by polyacrylamide sodium dodecyl sulfate gel electrophoresis [12.5% acrylamide concentration/0.625% bisacrylamide cross-linker concentration, with a linear gradient from 4 to 8 M urea (Shah and Milligan, 1994)] with 8-cm × 11-cm × 0.15-cm slab gels (Hoefer SE 280) at 20-mA constant current (ISCO power supply, model 595). For detection of PLCβ1, brain PAGs were collected in Tris buffer 50 mM (pH 7.4), 1 mM EDTA, 1 mM benzamidine, 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/μl leupeptin and 1 μg/μl pepstatin, and homogenized. After centrifugation, the supernatant was resolved by electrophoresis (gradient of 5-16% total acrylamide concentration/2.6% bisacrylamide cross-linker concentration).

The proteins were transferred (Mini-Trans-Blot electrophoretic transfer cell; Bio-Rad, Hercules, CA) to 0.2-μm polyvinylidene difluoride Trans-Blot membranes (Bio-Rad) using Towing buffer (25 mM Tris.HCl, 192 mM glycine, 0.04% sodium dodecyl sulfate, 20% methanol), by application of 70 V (200-300 mA) for 120 min. Unoccluded protein sites were blocked with 5% w/v non-fat dry milk (Blocker; Bio-Rad) in Tris-buffered saline for 1 hr at 37°C. The membranes were incubated with antibodies against Go subunits at 1:1,000 dilution in Tris buffered saline-0.05% Tween 20 at 6°C for 24 hr, and subsequently with a secondary antiserum [goat anti-rabbit IgG (H+L) horseradish peroxidase conjugate (BioRad, Hercules, CA #170-6515)] diluted 1:3,000 in Tris-buffered saline-0.05% Tween 20 for 3 hr. Antibody binding was detected using the substrate solution (1 mg/ml 3,3′-diaminobenzidine, 0.02% hydrogen peroxide, 0.4% nickel chloride in 0.1 M Trizma base buffer pH 7.2). A mixture of IgG monoclonal antibodies (Upstate Biotech, Lake Placid, NY, #05-164) diluted to 1:1,000 was used to detect PLCβ1. The IgGs were detected with a secondary antiserum [goat anti-mouse IgG (H+L) horseradish peroxidase conjugate (BioRad, #170-6516)] and visualized with Enhanced Chemiluminescence reagents (Amersham Corp., Buckinghamshire, UK). Immunoblots were analyzed by densitometry using an instrument with reflectance capabilities (BioRad, GS-700 Imaging Densitometer).

Antibodies and chemicals. The antisera used were raised against: anti Goα internal fragment [115-125: EEQGMPLEDLS] S/1 (Sánchez-Blázquez et al., 1993, 1995). Anti Gαα (371729-Q) and anti Gαα (371752-Q) were obtained from Calbiochem-Novabiochem Corporation (La Jolla, CA). Anti Gαα (GC/2, NEI-804) and anti Gαα (QL, NEI-809) came from Du Pont-New England Nuclear Research Products (Boston, MA). Anti-PLCβ1 (#05-164) monoclonal antibodies were obtained from Upstate Biotechnology. Human β-endorphin, DSLET, DPDPE and [d-Ala²]deltorphin II were purchased from Peninsula Laboratories Europe (Merseyside, UK).

Results

Changes in Go-like immunoreactivity after in vivo administration of oligodeoxynucleotides. Ten minutes after i.c.v. injection of the fluorescein-labeled ODN, fluorescence was detected in the periaqueductal region (fig. 1). Subsequently, and in a time-dependent fashion, these signals entered from the cerebral aqueduct and spread through the neural tissue. For immunodetection, the mice were killed 6 days after starting the subchronic administration of the ODNs and P2 membranes from PAG were obtained and resolved by polyacrylamide sodium dodecyl sulfate gel electrophoresis. In the absence of urea, the anti Gαα and anti Gαα appear to recognize a single, 42 kDa, polypeptide. Similarly, the anti Gα antisemur labeled a single band of 39 kDa (not shown). Gαα and G11α subunits could be resolved with a linear gradient of 4 to 8 M urea, G11α showing a greater electrophoretic mobility than Gαα subunits (fig. 2, upper panel). An identical approach was utilized to separate Gαα from Gαα in immunoblots (Mullaney and Milligan, 1990) (fig. 2, upper panel). The ODNs corresponding to mRNA of Ga subunits reduced the extent of labeling in immunoblots. Molecular weight markers are not indicated when urea was used, because this procedure alters the migration of the protein standards used. The diminishing effect of ODNs to mRNAs of Gαα and Gαα subunits on the target proteins has already been reported (Sánchez-Blázquez et al., 1995).

After resolving the soluble phase of mouse PAG by SDS-PAGE, the mixture of monoclonal antibodies raised against PLCβ1 recognized a protein of about 150 kDa (Suh et al., 1988). When mice had been treated with an ODN-PLCβ1 the intensity of labeling was reduced (fig. 2, bottom). This treatment showed no cross-effect on the immunoreactivity associated to Gαα, Gαα or G11α subunits in neural membranes (data not shown).

Effects of in vivo administration of ODNs and antibodies to Ga subunits on supraspinal analgesia induced by opioids binding delta opioid receptors. Mice that received i.c.v. injections of saline, the random oligo or ODNs to Ga subunits, responded in the analgesic test with basal latencies comparable to those of noninjected (naïve) mice. The analgesic substances produced similar effects in
mice that received i.c.v. injections of the vehicle, the random oligo, or in naive animals. Thus, the responsiveness of mice in the antinociceptive test was not altered by the experimental procedure alone.

The subchronic administration of ODNs to $G_{11}\alpha$, $G_q\alpha$, and $G_{11}\alpha$ subunits was followed by a significant decrease in the antinociceptive potency of the $\delta$-receptor agonists DPDPE and [D-Ala$^2$]deltorphin II (fig. 3). The impairment of $G_{11}\alpha$ function led to a weaker analgesic response to DPDPE, whereas, the effect of [D-Ala$^2$]deltorphin II was unaltered in these mice. The antisense ODN to $G_q\alpha$ subunit-mRNA produced opposite effect; DPDPE-induced antinociception was unchanged although the activity of the selective agonists of $\delta$-opioid receptors [D-Ala$^2$]deltorphin II and DSLET, as well as that exhibited by the endogenous opioid peptide $\beta$-endorphin, clearly diminished (figs. 3 and 4).

In a parallel set of experiments, 2 µg of affinity-purified IgGs to various $G\alpha$ subunits were i.c.v.-injected into the mice. Control animals received 2 µg of protein A-purified IgGs (preimmune serum) in the same volume. The supraspinal....
Antinociceptive activity of the opioid agonists was assessed 24 hr later. As previously described, i.c.v. administration of preimmune IgGs did not alter the response of the animals to the analgesic effect of opioids (Sánchez-Blázquez and Garzón, 1993; Sánchez-Blázquez et al., 1993, 1995). Immune IgGs heated at 100°C for 10 min lacked activity on opioid-induced antinociception (data not shown). However, a single administration of the antibodies to the G\textsubscript{a}\text{subunits} reproduced the effects of sub-chronic ODNs. I.c.v. injection of anti-G\textsubscript{a}\text{Gi2}, anti-G\textsubscript{a}\text{Gi3}, anti-G\textsubscript{a}\text{Go1/o2} and anti-G\textsubscript{a}\text{Gq/11} antibodies reduced the antinociceptive activity of the opioids binding to delta opioid receptors (fig. 5). The administration of anti-G\textsubscript{a}\text{Gq} IgGs only impaired the effect of the delta-2-agonist [D-Ala\textsuperscript{2}]deltorphin II.

**Agonist-antagonist activity of delta-selective ligands in ODN-treated mice.** In mice undergoing treatment with the random ODN the analgesia obtained by the combination of DPDPE and [D-Ala\textsuperscript{2}]deltorphin II was significantly higher than the value of either agonist when acting alone (fig. 6). After reducing the expression of G\textsubscript{a}\text{Go1}, DPDPE antagonized [D-Ala\textsuperscript{2}]deltorphin II in its capacity to produce an analgesic effect (fig. 6). After administration of ODN-G\textsubscript{a}, [D-Ala\textsuperscript{2}]deltorphin II did not alter the antinociceptive potency of DPDPE, a result suggesting the existence of distinct binding sites for these two agonists. In agreement with this, after the in vivo down-regulation of delta-2 receptor expression by ODN-delta\textsubscript{2-29} (Lai et al., 1994; Bilsky et al., 1996; Sánchez-Blázquez et al., 1997; Rossi et al., 1997), the antinociception induced by the selective agonist of delta-2 receptors [D-Ala\textsuperscript{2}]deltorphin II was strongly reduced. Interestingly, [D-Ala\textsuperscript{2}]deltorphin II did not interfere with the antinociception evoked by DPDPE in these animals (fig. 6).

**Effect of in vivo administration of ODNs to phospholipase Cβ1 on the supraspinal analgesia induced by delta agonists in mice.** The effect of ODN-PLCβ1 was evaluated in vivo. Mice subjected to subchronic administration of the ODN to PLCβ1 showed an impaired analgesic response to [D-Ala\textsuperscript{2}]deltorphin II, DSLET and β-endorphin. Notwithstanding, in these mice the antinociception induced by DPDPE was preserved and [D-Ala\textsuperscript{2}]deltorphin II did not antagonize DPDPE (fig. 7). The effect of agonists binding mu-opioid receptors, DAMGO and morphine, was not altered by treatment with the antisense ODN to phospholipase Cβ1 (not shown).

**Discussion**

Pharmacological studies (Mattia et al., 1991; Sofuoglu et al., 1991a, b; Portoghese et al., 1992; Vanderah et al., 1994)
and antisense ODN strategies (Lai et al., 1994; Bilsky et al., 1996; Sánchez-Bláquez et al., 1997) have convincingly demonstrated the existence of subtypes of the delta opioid receptors. However, only one molecular form for this opioid receptor has been described so far (Evans et al., 1992; Kieffer et al., 1992; Yasuda et al., 1994). In vivo studies using antibodies (Sánchez-Bláquez and Garzón, 1993; Sánchez-Bláquez et al., 1993, 1995) or antisense ODNs (Raffa et al., 1994; Sánchez-Bláquez et al., 1995; Standifer et al., 1996) directed to Ga subunits of transducer proteins known to inhibit adenylyl cyclase activity, it was found that the blocking of Gi2 or Gi3 subunits, but not Gi1 or Gx/z, significantly reduced delta-mediated supraspinal antinociception in mice. Although these treatments effectively differentiated mu- and delta-mediated antinociception, they did not discriminate between the pharmacologically defined subtypes of delta opioid receptors.

The coupling of delta receptors to various effector systems (e.g., phospholipase C and type II adenylyl cyclase) has been documented in a series of in vitro studies (Tsu et al., 1995). The observed impairment of delta-mediated supraspinal antinociception by antisense ODNs complementary to Go1 and Gq subunits, indicates that distinct signaling pathways associated with phosphoinositide cascades are involved in this opioid effect. The Gi2, Gi3, Go2 and G11 proteins are activated by both subtypes of delta opioid receptors in the production of supraspinal antinociception (Raffa et al., 1994; Sánchez-Bláquez et al., 1995; Standifer et al., 1996; present work). Go1 proteins are regulated selectively by delta-1 receptors whereas Gq seems to be linked solely to the effect of delta-2-agonists (fig. 8).

The selective regulation of different classes of G proteins by the delta-agonists in the production of antinociception, supports the proposed existence of two separate subtypes of delta opioid receptor. It might also be feasible that after binding a unique delta opioid receptor, certain agonists acti-
receptor gene are also consistent with the existence of closely related molecular forms of this receptor (Rossi et al., 1997; Sánchez-Blázquez et al., 1997). In mice whose delta receptor gene has been disrupted by gene targeting, the capacity of both DPDPE and [D-Ala²]deltorphin II to produce analgesia was strongly but not completely reduced (Zhu et al., personal communication). Therefore, at the present time, it cannot be concluded whether the delta receptor subtypes arise as splice variants from a unique gene, or come from separate genes.

In this study, it was shown that besides the Gi family of G-transducer proteins, delta receptors are associated with Go and Gq classes in the production of supraspinal antinociception in the mouse. Jin et al. (1994) have reported activation of phosphoinositide cascades via delta receptors and the subsequent mobilization of calcium from inositol 1,4,5-triphosphate sensitive stores in neuroblastoma x glioma 108-15 cells. In Ltk⁻ fibroblasts transfected with the cloned delta receptor, DPDPE was able to stimulate phosphoinositide-specific phospholipase C through a pertussis toxin-sensitive G protein (Tsu et al., 1995). The inhibition of the a-subunits of Gi and Go proteins reduced the degree of analgesia evoked by DPDPE. These findings indicate that pertussis toxin-sensitive G proteins are implicated mainly in the physiological effects of this compound. The G proteins activated by the pharmacologically defined delta-1 receptor in the production of supraspinal antinociception (fig. 3) are in accordance with observations made when using membranes of human neuroblastoma SH-SYSY cells. In these cells, which exhibit mu and delta receptors, DPDPE, after binding selectively to a subunit of this receptor, Gq, reduced the effect of [D-Ala²]deltorphin II antagonism of DPDPE activity. These observations indicate that, the epsilon opioid receptors, DPDPE, which is more effective than DPDPE in activating Gl and Gq classes in the production of supraspinal antinociception. Adapted from Sánchez-Blázquez et al. (1995) and present work.

![Fig. 8. Assignment of G proteins to delta-1 and delta-2-opioid receptors in the production of supraspinal analgesia. The solid lines denote significant reductions in analgesic potency in mice undergoing treatment with ODNs to the corresponding Go subunits. Dotted lines indicate G proteins where one of the subtypes seems to lack regulation. G proteins without arrows are those regulated by neither type of delta receptors in the production of supraspinal antinociception. Adapted from Sánchez-Blázquez et al. (1995) and present work.](image-url)
induced activation of G11 proteins in the production of supraspinal antinociception appears not to be related to PLCβ1 as no reduction was found for this agonist in mice treated with the ODN to this enzyme. Whether DPDPD leads to stimulation of PLCβ2 and/or PLCβ3 isoforms remains to be seen.

In summary, the supraspinal antinociception produced by agonist-activated delta receptors is mediated by G proteins of the Gq/11 family as well as by Gi- and Go-transducer proteins. The present work provides further evidence favoring the existence of delta-1- and delta-2-opioid receptors. G01 proteins seem to be selectively activated by delta-1 receptors, whereas delta-2 receptors show preference for the pertussis toxin-insensitive Gq proteins in this effect. It might therefore be concluded that delta receptors trigger at least two signaling cascades, adenyl cyclase and phospholipase C, in the production of analgesia.

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References


Send reprint requests to: Dr. Pilar Sánchez-Blañquez, Instituto Cajal, CSIC, Avenida Doctor Arce 37, E-28002, Madrid, Spain.