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The role of the G protein γ_2 subunit in opioid antinociception in mice

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Abstract

We examined the role of the γ_2 subunit of G proteins ($G\gamma_2$) in the antinociception produced by c[D-Pen^2,D-Pen^5]enkephalin (DPDPE) in mice. DPDPE produced 84.0 ± 9.0% antinociception in vehicle-treated mice. After intracerebroventricular (i.c.v.) treatment with an antisense phosphorothioate oligodeoxynucleotide to the $G\gamma_2$ subunit, DPDPE-mediated antinociception decreased to 24.4 ± 7.4%. The mismatch phosphorothioate oligodeoxynucleotide-treated mice showed 65.1 ± 10.3% antinociception, while the missense phosphorothioate oligodeoxynucleotide-treated mice showed 76.4 ± 23.6% antinociception by DPDPE. The reduction of analgesia in antisense phosphorothioate oligodeoxynucleotide-treated mice was significant in comparison with vehicle-treated ($P < 0.001$), mismatch phosphorothioate oligodeoxynucleotide-treated ($P < 0.01$) and missense phosphorothioate oligodeoxynucleotide-treated ($P < 0.05$) mice. These results suggest that the G protein γ_2 subunit is involved in the transduction pathway leading to antinociception by DPDPE.

Keywords: G protein γ_2 subunit; DPDPE (c[D-Pen^2,D-Pen^5]enkephalin); Antinociception

1. Introduction

The synthetic cyclic derivative of the endogenous opioid agonist, enkephalin, c[D-Pen^2,D-Pen^5]enkephalin (DPDPE), has been shown to produce supraspinal analgesia in mice. The analgesia is antagonized by naltrexone, indicating the involvement of opioid receptors. Previous reports have established the role of $G_{i/o}$ proteins in DPDPE-mediated analgesia, as the supraspinal analgesia induced by DPDPE was blocked by pertussis toxin (Sanchez-Blazquez and Garzon, 1992) and by antisense phosphorothioate oligodeoxynucleotides to the α subunits of $G_{i/o}$ proteins (Standifer et al., 1996). However, the molecular mechanisms mediating opioid analgesia have not been established unequivocally.

Agonist binding to opioid receptors has been shown to lead to the inhibition of cAMP formation, the activation of inwardly rectifying K^+ channels (GIRKs), the inhibition of voltage-sensitive Ca^{2+} channels, the stimulation of phospholipase Cβ isoenzymes, and the activation of the mitogen-activated protein (MAP) kinase pathway (Quock et al., 1999). The inhibition of cAMP formation by opioid receptors is thought to be mediated directly by the activated α subunit of the $G_{i/o}$ proteins. However, recent evidence indicates the involvement of Gβγ subunits of the heterotrimeric G proteins in the regulation of the GIRK and voltage-sensitive Ca^{2+} channels, certain adenylyl cyclases, the pertussis-toxin-sensitive regulation of PLCβ, and the activation of the MAP kinase pathway (Clapham and Neer, 1997).

Genes encoding 20 different α-, six β- and 12 γ subunits of the G proteins have been identified in mammalian systems (Hamm, 1998). The rules determining the specificity of the interaction between receptors and G protein subunits or between effectors and G protein subunits are not known. In intact cells using antisense oligodeoxynucleotides (Kleuss et al., 1993) or ribozymes (Wang et al., 1997), both the receptor–βγ subunit and βγ subunit–effector interactions were shown to be highly specific.

The amino acid sequences of the β subunits are highly homologous, while the sequences of the γ subunits are

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more divergent (27–75% homology). Consequently, the specificity of receptor–$\beta\gamma$ subunit interactions is thought to be conferred by the γ subunit of the complex. The γ_2 subunit is the most abundant G protein (-subunit in brain (Betty et al., 1998). Therefore, we selected the $G\gamma_2$ subunit to test the role of the $\beta\gamma$ subunit regulated second messenger pathway(s) in the production of analgesia in mouse brain.

In this report, we investigated the involvement of the $G\gamma_2$ subunit in antinociception mediated by DPDPE in mice after intracerebroventricular (i.c.v.) treatment with antisense-, mismatch- or missense phosphorothioate oligodeoxynucleotides to the $G\gamma_2$ subunit. We found that the treatment with antisense phosphorothioate oligodeoxynucleotide against the $G\gamma_2$ subunit produced a significant reduction in antinociception mediated by DPDPE as compared to vehicle-, mismatch phosphorothioate oligodeoxynucleotide- and missense phosphorothioate oligodeoxynucleotide treatments.

2. Materials and methods

We measured antinociception 24 h after the last oligodeoxynucleotide administration using the 55°C warm water tail-flick test. Male ICR mice were used for this study. All compounds were injected by the i.c.v. route into mice lightly anesthetized with ether. An initial latency time was determined before the mice were included in the study. Mice not responding within 5 s were excluded from further experiments. A phosphorothioate-modified antisense oligodeoxynucleotide, corresponding to nucleotides 1–24 in the open reading frame of the mouse $G\gamma_2$ subunit cDNA, was synthesized (Integrated DNA Technologies, Coralville, IA). The sequence of the antisense phosphorothioate oligodeoxynucleotide was: 5′-GCT-GGC-GGT-GTT-GTT-GCT-GGC-CAT-3′. The mismatch phosphorothioate oligodeoxynucleotide was produced by switching two bases of each codon (5′-GTC-GCG-GTG-TGT-TGT-CGT-GCG-ACT-3′) and the missense phosphorothioate oligodeoxynucleotide was produced by scrambling and switching codon pairs (5′-CGG-TCG-TTG-TGG-TGC-TTG-TCA-CGG-3′). The mismatch phosphorothioate oligodeoxynucleotide and missense phosphorothioate oligodeoxynucleotide were used as controls in this study. The BLAST Search program (NCBI, Bethesda, MD) was used to determine the homology of the antisense phosphorothioate oligodeoxynucleotide and mismatch phosphorothioate or missense phosphorothioate oligodeoxynucleotides to sequences deposited in GenBank. The antisense phosphorothioate oligodeoxynucleotide sequence did not produce significant alignment with any mammalian genes except the species homologues of $G\gamma_2$, and neither the mismatch- nor the missense phosphorothioate oligodeoxynucleotides produced significant alignment with any mammalian nucleotide sequences in GenBank. All of the oligodeoxynucleotides were dissolved in distilled water and injected by i.c.v. route into the right lateral ventricle of each mouse. Each mouse received 10 μg of antisense- or mismatch- or missense phosphorothioate oligodeoxynucleotides in 5 μl volume once daily at 24 h intervals for 2 days. The control mice were injected at the same time with the same volume of vehicle (distilled water) instead of the oligodeoxynucleotides. The basal latency of antinociception was measured before the administration of DPDPE. Mice with basal latency longer than 5 s were excluded from further experiments. Each mouse received 30 nmol DPDPE (synthesized in Dr. V.J. Hruby's laboratory) prepared in 5 μl of distilled water by the i.c.v. route and the latency was measured 10 min later. In preliminary experiments, the peak latency response was determined to be 10 min after the administration of DPDPE. Mice were assigned 100% antinociception when not responding within 15 s and their tails were removed from the water to avoid tissue damage. Antinociception was calculated by the following formula: % antinociception = (test latency − basal latency) × 100/(15 s − basal latency).

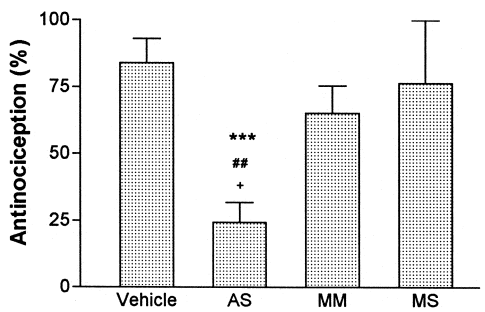

Fig. 1. The role of the G protein γ_2 subunit for DPDPE-mediated antinociception in mice. The bar graph represents the mean ± standard error of the mean for 12 mice in the vehicle group, 12 mice in the antisense phosphorothioate oligodeoxynucleotide-treated group (AS), eight mice in the mismatch phosphorothioate oligodeoxynucleotide-treated group (MM) and four mice in the missense phosphorothioate oligodeoxynucleotide-treated group (MS). Ten micrograms of each oligodeoxynucleotide prepared in 5 μl of distilled water (vehicle) was injected by the i.c.v. route to each mouse once daily for 2 days. The same volume (5 μl) of distilled water was injected into the control animals (vehicle group) by the i.c.v. route. The peak latency response was measured at 10 min after the i.c.v. administration of DPDPE (30 nmol) prepared in 5 μl of distilled water. DPDPE produced 84.0 ± 9.0% antinociception in vehicle-treated mice. In contrast, DPDPE produced 24.4 ± 7.4% antinociception in mice treated with antisense phosphorothioate oligodeoxynucleotide to the $G\gamma_2$ subunit. However, mice treated with either mismatch phosphorothioate oligodeoxynucleotide or missense phosphorothioate oligodeoxynucleotide exhibited 65.1 ± 10.3% and 76.4 ± 23.6% antinociception following i.c.v. injected DPDPE, respectively. The differences between antisense phosphorothioate oligodeoxynucleotide-treated mice and vehicle-treated mice ($^{***}P < 0.001$), mismatch phosphorothioate oligodeoxynucleotide-treated mice ($^{\#\#}P < 0.01$) or missense phosphorothioate oligodeoxynucleotide-treated mice ($^{+}P < 0.05$) were determined to be significant by one-way ANOVA, followed by Newman–Keuls multiple comparison test.

3. Results and discussion

DPDPE produced 84.0 ± 9.0% antinociception in vehicle-treated mice. In contrast, DPDPE produced 24.4 ± 7.4% antinociception in mice treated with antisense phosphorothioate oligodeoxynucleotide to the $G\gamma_2$ subunit. However, mice treated with either mismatch- or missense phosphorothioate oligodeoxynucleotide exhibited 65.1 ± 10.3% and 76.4 ± 23.6% antinociception, respectively (Fig. 1). The differences between antisense phosphorothioate oligodeoxynucleotide-treated mice and vehicle-, mismatch phosphorothioate oligodeoxynucleotide-, or missense phosphorothioate oligodeoxynucleotide-treated mice were determined to be significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively, by one-way ANOVA, followed by Newman–Keuls multiple comparison test. In conclusion, these results indicate that the knockdown of the G protein γ_2 subunit in mouse brain causes a significant reduction in DPDPE-induced analgesia. Our findings suggest that the G protein γ_2 subunit is involved in the antinociception produced by DPDPE.

Acknowledgements

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