

**EFFECTS OF SPONTANEOUS AND NALOXONE-PRECIPITATED MORPHINE WITHDRAWAL ON G(q/11) AND G(12) PROTEIN LEVEL IN RAT BRAIN**


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**Introduction**

Morphine, an agonist of opioid receptors, is a potent analgesic drug with known addictive properties. Guanine nucleotide-binding proteins (G proteins) composed of Gα and Gβγ subunits play critical role in brain signal transduction. G proteins, attached to the cell plasma membrane, that connect receptors to effectors and thus to intracellular signaling pathways. On the basis of sequence similarity, the Gβγ subunits have been divided into four families: Gαq, Gαi/o, Gαq/i11, Gαq/i12 (Tab. 1). Opioid receptors are coupled with the Gαq/o family. Recently, we have found that chronic treatment with morphine affects the mRNA level of Gαq/i11 and Gαq/i12 subunits, which are known to be linked to other opioid receptors.

The identification of morphine-induced changes in the expression of Gαβγ subunits is of critical importance for understanding of addictive behavior.

**Tab. 1. Heterotrimetric G-protein α subunit families and their effect**

<table>
<thead>
<tr>
<th>G protein (q) subunit family</th>
<th>Effect of activation</th>
<th>Receptor coupled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gα(q) αq-Gq or αq-βγ</td>
<td>stimulation AC, regulation Ca²⁺ channel</td>
<td>DI, D₂, D₅R, HT₁, 5-HT₆, 7, 8, 12, 15, 16</td>
</tr>
<tr>
<td>Gα(i)/o αi-Gi or αi-βγ</td>
<td>stimulation K⁺ and Ca²⁺ channel, activation Gαq protein phosphatase</td>
<td>Gαi/i2, M₁, M₂, D₂, D₃, mGluR₁, 7, 8, OR</td>
</tr>
<tr>
<td>Gα(q) αq-Gq or αq-βγ</td>
<td>activation of PLC β</td>
<td>Gα₂, 6, 9, 11, 12</td>
</tr>
<tr>
<td>Gα(q)/i12(i13) αi12-i13</td>
<td>activation of Bhc signaling</td>
<td>βγα₁, α₁, 2, 3, 5, 11, 12</td>
</tr>
</tbody>
</table>

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**Aim**

The aim of current study was to investigate whether the morphine withdrawal changes the protein level of Gαq/i11 and Gαq/i12 in rat prefrontal cortex.

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**Methods**

**Animals and Treatment**

Male Wistar rats were injected with increasing doses of morphine (10–50 mg/kg, i.p., twice daily, 14 days). Half of them were injected only with morphine and decapitated 3 hrs after last dose or withdrawn from the drug for 48 hrs. Other two groups of animals were given the opioid receptor antagonist, naloxone: one dose of 20 mg/kg, 1 h after the last morphine injection or 3 doses (10 mg/kg) at 15 min, 2 and 46 hrs after the morphine cessation. Animals were decapitated at 2 hrs after the last naloxone injection.

**Behavioural Experiment**

Locomotor activity, defined as a distance travel (DT), was measured in Opto-Violinc cages (Columbus Instruments, USA).

**Assessment of protein expression:**

*Proprotein Cortex was immediately isolated from the brain and tissues were frozen in liquid nitrogen.

Western-blotting technique for Gαq/i11 and Gαq/i12 using specific rabbit 1st polyclonal Ab and anti-rabbit 2nd Ab conjugated to POD was performed. Lumiled chromatography was densitometrically measured by fluorescent (FUJI-LAS 1000, Fuji, Japan).

*Values were recalculated versus actin levels which served as a reference protein and loading control.

**Statistics**

Behavioral study – values represent the means ± S.E.M. of 8 animals.

Biochemical study – values represent the means ± S.E.M. of 6–14 animals.

**Statistical analysis was carried out using two way analysis of variance followed by the Fisher’s LSD test.**

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**Results**

- Morphine enhanced locomotor activity (ca. 8-fold, p < 0.01) and naloxone (one dose of 20 mg/kg) abolished the effect of morphine (Fig. 1).

- Morphine treatment induced the increase of Gαq/i11 protein level by 42% vs saline control (p<0.001) and the effect was abolished by naloxone (Fig. 2A).

- The Gαq protein expression, unaffected by morphine, was increased after the blockade of opioid receptors by 58% (p<0.001) (Fig. 3A).

- The Gαq and Gαq protein expression was similarly attenuated after spontaneous and naloxone-precipitated withdrawal from morphine (by 20 % and 30 % vs saline) (Fig. 2B and 3B).

- None of treatment procedures affected the expression of Gαq/i12 subunit (Fig. 4).

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**Fig. 1. Locomotor activity of rats after naloxone-precipitated (A) and spontaneous (B) morphine withdrawal**

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**Fig. 2. Effects of naloxone-precipitated (A) and spontaneous (B) morphine withdrawal on the Gαq/i11 protein level in the rat prefrontal cortex**

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**Fig. 3. Effects of naloxone-precipitated (A) and spontaneous (B) morphine withdrawal on the Gαq/i12 protein level in the rat prefrontal cortex**

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**Fig. 4. Effects of naloxone-precipitated (A) and spontaneous (B) morphine withdrawal on the Gαq/i12 protein level in the rat prefrontal cortex**

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**Conclusions**

- Treatment with morphine and withdrawal of the drug induce opposite effects on Gαq/i12 expression.

- The blockade of opioid receptors during presence of morphine in the brain modulates the expression of Gαq/i11 and Gαq/i12 proteins.

- The withdrawal-induced changes in Gαq/i11 and Gαq/i12 are independent of opioid receptors.

Although, the functional meaning of the phenomenon requires further studies, the results suggest that Gαq/i11 and Gαq/i12 may be involved in the development of tolerance and/or dependence upon morphine.

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**References**