Morphine + NLX
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Ultra-low-dose NLX blocks chronic morphine-induced Go to Gs switch in organotypic striatal slice cultures

INTRODUCTION

Drug treatment of striatal slices

To induce morphine tolerance, two striatal slices on one incubate in each of the 12-well culture dish (12 slices in 8 wells) were exposed to 10 µM morphine for 1 hr, twice daily for 7 days. To assess the effect of ultra-low-dose NLX on the chronic morphine-mediated signaling switch, 12 separate slices were exposed to 10 µM morphine + 10 µM NLX. As control, slices were exposed to 10 µM morphine or vehicle alone or to vehicle. For each drug culture, medium was removed, 1 µl tissue lysis was gently rinsed twice with warm phosphate-buffered saline (32°C, pH 7.2) just before introducing in respective drug concentrations in 0.1% heat inactivate medium culture. Brain slices were reselected for cultures with normal after each drug exposure.

Co-immunoprecipitation of G proteins and MOR

Tissues were harvested 16 hr after the last drug exposure by centrifugation. For determination of MOR-G protein coupling, brain slices were homogenized to generate synaptic membranes. Synaptic membranes (400 µg) were incubated with either 1 µM morphine or Ketanserin-Ringer solution for 10 min before being solubilized in 250 µl of immunoprecipitation buffer (25 µl HEPES, pH 7.5, 200 mM NaCl, 1 mM EDTA, 50 µg/ml leupeptin, 10 µg/ml aprotonin, 2 µg/ml soybean trypsin inhibitor, 0.1 mM PMSF, and moderate of protein phosphatase inhibitors). Following centrifugation, striatal membrane lysates were immunoprecipitated with immobilized anti-Gi and with immobilized protein G-agarose beads. The level of MOR in anti-Gi or anti-Gα antibodies was determined by Western blotting using specific anti-MOR antibodies.

ulAMF production assay

Since Go is known to inhibit adenylate cyclase and Gi is known to stimulate this enzyme, the chronic morphine-induced switch in MOR signaling from Co to Gi in Go slices above should increase adenylate cyclase activity and resulting levels of cAMP. As expected, chronic morphine exposure increased cAMP accumulation by 24%, compared to levels in slices exposed only to culture medium, after receptor activation by DAMGO (Fig. 3). Chronic morphine exposure also attenuated the DAMGO-mediated inhibition of forskolin-stimulated cAMP accumulation, reducing the inhibition from the 100 µM inhibitor level by 23% with ultra-low-dose NLX-treated slices to 7%. Both chronic morphine-induced effects on cAMP accumulation were blocked by co-treatment with ultra-low-dose NLX. Exposure to NLX alone had no effect on ulAMF accumulation.

RESULTS

We previously demonstrated the G protein coupling switch in striatal and spinal chord tissue harvested from rats treated with chronic morphine (Wang et al., 2005). In this present study, chronic morphine exposure blocks MOR coupling, restricting the parts of the schedule, also resulted in a Go to Gi coupling switch by MOR (Fig. 2). We determined the effect of ultra-low-dose NLX on the chronic morphine-induced signaling switch, 12 separate slices were exposed to 10 µM morphine + 10 µM NLX. As control, slices were exposed to 10 µM morphine or vehicle alone or to vehicle. For each drug culture, medium was removed, 1 µl tissue lysis was gently rinsed twice with warm phosphate-buffered saline (32°C, pH 7.2) just before introducing in respective drug concentrations in 0.1% heat inactivate medium culture. Brain slices were reselected for cultures with normal after each drug exposure.

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METHODS

Organic dyes

Striatal slices were exposed to 10 µM morphine + 10 pM NLX for 1 hr, twice daily for 6 days. As control, slices were exposed to 10 µM morphine or vehicle alone or to vehicle. For each drug culture, medium was removed, 1 µl tissue lysis was gently rinsed twice with warm phosphate-buffered saline (32°C, pH 7.2) just before introducing in respective drug concentrations in 0.1% heat inactivate medium culture. Brain slices were reselected for cultures with normal after each drug exposure.

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REFERENCES