EFFECTS OF MORPHINE ON NOP RECEPTOR GENE EXPRESSION IN SH-SY5Y CELLS

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The neuropeptide nociceptin (or orphanin FQ) is the endogenous ligand for the ORL-1 receptor, recently referred to as NOP. This receptor presents marked structural analogies with the three different opioid receptors, nevertheless it isn't able to interact with the ligands for such receptors (1). The pharmacological characterization of this new neuronal system allowed to suggest that nociceptin acts as a functional antagonist towards the endogenous opioid system.
At this regard, it has been recently observed that morphine, interacting with the μ opioid receptor, modulates pronociceptin gene expression in selective areas of rat brain (2), suggesting the involvement of this system in the mechanisms underlying the development of morphine tolerance.
With the aim of better characterizing the interplay between nociceptin/NOP and opioid systems and in order to investigate the possibility of a cross-talk between μ opioid receptor and NOP receptor, the effects of morphine on human NOP gene expression were examined in SH-SY5Y cells.
This human neuroblastoma expresses hNOP receptor as well as μ and δ opioid receptors. The cells were exposed to 10 μM morphine, according to a detailed time-course (5, 24, 48 hours and 5 days); total RNA was extracted and quantified by Northern Analysis using a cDNA probe, that was complementary to human NOP receptor sequence. It consisted of Pst I and Hind III digested fragment of the pBlueScript plasmid. In this conditions, the hybridization yielded a main band corresponding to mature mRNA and two other isoforms (probably due to alternative splicing). Exposure to morphine produced a significant decrease of hNOP receptor mRNA levels after 5 hours (53.92 ± 3.01 % vs controls 100.00 ± 10.37 %, $p<0.05$, Newman-Keuls test), that returned to control values after 24 and 48 hours (99.73 ± 11.56 % e 84.08 ± 8.05% vs controls, rispectively). After 5 days, a significant decrease (50.32 ± 7.12 % vs controls, $p<0.01$, Newman-Keuls test) was observed.
These results clearly show that morphine is able to inhibit NOP gene expression in SH-SY5Y cells and that the mechanisms activated by chronic occupation of μ opioid receptor can contribute to the regulation of NOP receptor biosynthesis. Furthermore, they confirm the existence of interactions between opioid and nociceptin/NOP systems, thus suggesting a real cross-talk between μ and NOP receptor.
Finally, the data reported in this study both confirm the results previously obtained in the rat CNS and strengthen the hypothesis of a functional antagonism between these two neuronal systems; in addition they suggest the involvement of nociceptin/NOP system in the cellular mechanisms, underlying the opioid tolerance.

References

1. Meunier JC, Mollereau C, Toll L et al. *Nature* 377, 532-535, 1995.

2. Romualdi P, Landuzzi D, D'Addario C, Candeletti S, *NeuroReport* 13, 645-648, 2002.
