

## ROLE OF THE TYPE 2 AND 3 INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR IN MUSCARINIC ANTINOCICEPTION IN MICE

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The importance of the receptor-mediated activation of the PLC-IP<sub>3</sub> pathway in the cholinomimetic antinociceptive effect was evidenced not only by the inhibition of the 1,4,5-trisphosphate (IP<sub>3</sub>) synthesis induced by LiCl but also the antagonism exerted by heparin a blocker of IP<sub>3</sub> receptors (IP<sub>3</sub>Rs) (1). IP<sub>3</sub>Rs are recognized as a protein family of tetrameric ligand-gated Ca<sup>2+</sup> channels which allow mobilization of intracellular Ca<sup>2+</sup> store. All three isoforms of IP<sub>3</sub>R are endowed with a molecular mass of about 300 kDa and share 60-70% aminoacid similarity (2). The aim of the present study was to investigate the role of type 1,2,3 IP<sub>3</sub>R in the intracellular mechanism of muscarinic antinociception at supraspinal level. To this purpose each IP<sub>3</sub> receptor subtype was inhibited by using antisense oligonucleotides (aODNs) targeting IP<sub>3</sub>R mRNA sequences which are unique in the mouse genome and therefore assure stringent target selectivity. The IP<sub>3</sub>R protein level reduction of approximately 30-50% produced by aODN administration for each receptor subtype was demonstrated by western blotting experiments. Intracerebroventricular (i.c.v.) pretreatment with an aODN complementary to the sequence of the type 2 IP<sub>3</sub>R (0.1-3 nmol per mouse) prevented the increase of pain threshold induced by physostigmine (0.15 mgkg<sup>-1</sup> s.c.) evaluated on hot-plate test in the presence of a thermal stimulus. Similarly, an aODN against type 3 IP<sub>3</sub>R (0.1-3 nmol per mouse) completely antagonized cholinergic antinociception. Dose-response curve of physostigmine was shifted to the right after IP<sub>3</sub>R2 and IP<sub>3</sub>R3 treatments. Conversely pretreatment with an aODN complementary to the sequence of the type 1 IP<sub>3</sub>R (0.1-5 nmol per mouse) did not modify analgesia induced by physostigmine. Mice undergoing treatment with aODNs did not show any impairment of the locomotor activity, spontaneous mobility and exploratory activity as revealed by the rota-rod and hole board tests. These results indicate a selective involvement of the type 2 and 3 IP<sub>3</sub>R in central cholinergic analgesia in mice whereas the type 1 IP<sub>3</sub>R appears not to play a prominent role in the physostigmine enhancement of pain threshold.

1. Galeotti N., Bartolini A. and Ghelardini C. (2003) *Neuropsychopharmacol.* 28: 888-897.
2. Taylor C.W., Genazzani A.A. and Morris S.A. (1999) *Cell Calcium* 26: 237-251.