

## SELECTIVE H<sub>3</sub> RECEPTOR ISOFORMS ACTIVATE AKT/GSK AXIS AND ERK IN RAT CORTICAL NEURONS

Chiara Mariottini<sup>1</sup>, Gerold Bongers<sup>3</sup>, Rob Leurs<sup>3</sup>, Silvia Fossati<sup>2</sup>, Alberto Chiarugi<sup>2</sup>, Patrizio Blandina<sup>2</sup> and M. Beatrice Passani<sup>2</sup>

<sup>1</sup>Dipartimento di Fisiologia, V.le Morgagni 65, Firenze, Italy

<sup>2</sup>Dipartimento di Farmacologia Preclinica e Clinica, V.le Pieraccini 6, Firenze, Italy

<sup>3</sup>Department of Medicinal Chemistry, Vrije Universiteit, Amsterdam, The Netherlands

The H<sub>3</sub> receptor (H<sub>3</sub>R) shows functional constitutive activity, polymorphisms in humans and rodents with a differential distribution of splice variants in the CNS, and potential coupling to different intracellular signal transduction mechanisms, such as inhibition of adenylate cyclase [1], MAPK phosphorylation [2]. Using RT-PCR we show that ED17 cortical neurons maintained in culture for 4, 7 or 11 days stably express H<sub>3</sub>R mRNA; furthermore cortical neurons express two out of the three functional H<sub>3</sub>R isoforms, namely A and C. Consistent with previously published data [2], the primers used recognise isoforms A, B and C in adult brains.

Incubation of cortical neurons with histamine (HA, 100 nM), the H<sub>3</sub>R agonists R- $\alpha$ -methylhistamine (RAMH, 100 nM) or imzepip (10 nM) induce a time dependent activation of the Akt/GSK3 $\beta$  axis that peaks at 60 min. Activation of Akt/GSK3 $\beta$  axis is inhibited by the H<sub>3</sub>R antagonist thioperamide (10  $\mu$ M) and by LY294002 (50  $\mu$ M) an inhibitor of PI3 kinase. We also demonstrate that the H<sub>3</sub>R agonist imzepip (10 nM) induces phosphorylation of ERK 1, 2 that reaches a maximum at 15 min. ERK activation was inhibited by the MEK inhibitor UO126 (10  $\mu$ M) and the H<sub>3</sub>R antagonist thioperamide.

H<sub>3</sub>R ligands affect cognition, the sleep-wake cycle, obesity and epilepsy, which are physiological and pathological conditions that are the main focus of research into the therapeutic potential of selective H<sub>3</sub>R ligands. Given the molecular and pharmacological heterogeneity of the H<sub>3</sub>R, it is important to understand if and which transduction cascades are associated to different H<sub>3</sub>R isoforms in light of more selective therapeutic treatments devoid of unwanted side effects.

1 Lovenberg TW., Roland BL., Wilson SJ., Jiang X., Pyati J., Huvar A., Jackson MR., Erlander MG. (1999) *Mol Pharmacol* 55(6):1101-7.

2 Drutel G., Peitsaro N., Karlstedt K., Wieland K., Smit MJ., Timmerman H., Panula P., Leurs R. (2001) *Mol Pharmacol* 59(1):1-8.