In vitro and in vivo pharmacological characterization of the novel neuropeptide S receptor ligands QA1 and PI1

C. Ruzza[1], V. Camarda[1], A. Rizzi[1], C. Trapella[2], R. Guerrini[2], R. K. Reinscheid[3], and G. Calo'[1]

[1]Dept. of Medical Science, Section of Pharmacology and National Institute of Neuroscience, University of Ferrara, 44121 Ferrara, Italy.
[2]Dept. of Chemical and Pharmaceutical Sciences and LTTA, University of Ferrara, 44121 Ferrara, Italy.
[3]Dept. of Pharmaceutical Sciences, University of California Irvine, Irvine, CA 92697, USA.

Neuropeptide S (NPS) selectively binds and activates a previously orphan GPCR, now named NPSR. Biological functions modulated by this peptidergic system include anxiety, arousal, locomotion, food intake, learning and memory, pain and drug addiction (Guerrini et al. 2010). In the present study the pharmacological activity of the novel NPSR ligands QA1 (Melamed et al. 2010) and PI1 (Trotter et al., 2010) was investigated in vitro and in vivo. In vitro QA1 and PI1 were tested in calcium mobilization studies performed in HEK293 cells expressing the recombinant mouse ($HEK293_{mNPSR}$) and human ($HEK293hNPSR_{Ile107}$ and $HEK293hNPSR_{Asn107}$) NPSR receptors. NPS caused a concentration dependent mobilization of intracellular calcium in the three cell lines with high potency (pEC_{50} 8.73 - 9.14). In inhibition response curve and Schild protocol experiments the effects of NPS were antagonized by QA1 and PI1. QA1 behaved as a insurmountable antagonist showing very high potency (pK_B 9.60 - 10.17). Conjection experiments demonstrated that the behaviour of QA1 is likely due to the hemiequilibrium conditions characteristic of the calcium assay rather than to a non competitive interaction with NPSR. PI1 displayed a competitive type of antagonism and lower values of potencies (pA_2 7.74 - 8.95). In vivo in mice NPS (0.1 nmol, i.c.v.) elicited robust arousal promoting action in the righting reflex (RR) assay and stimulant effects in the locomotor activity (LA) test. QA1 (30 mg/kg) was able to partially counteract the arousal promoting NPS effects, while PI1 was inactive in the RR test. In the LA test QA1 and PI1 only poorly blocked the NPS action. The present data demonstrated that QA1 and PI1 act as potent NPSR antagonists in vitro, however their usefulness for in vivo investigations in mice seems to be limited probably by pharmacokinetic reasons.

Guerrini et al. (2010) *Med Res Rev*. 30:751-77.
Melamed et al. (2010) *Bioorg Med Chem Lett*. 20:4700-3
Trotter et al. (2010) *Bioorg Med Chem Lett.* 20:4704-8.