Prokineticin receptor antagonists: pharmacological characterization

A.Cappiello[1], V. Marconi[1], R. Lattanzi[1], D. Maftei[1], L.A. Giancotti[1], G. Balboni[2], C. Ambrosio[3], L. Negri[1]

[1]Dept. of Physiology and Pharmacology 'Vittorio Erspamer', Sapienza University of Rome, Italy
[2]Dept. of Toxicology, University of Cagliari, Italy
[3]Dept. of Pharmacology, Istituto Superiore di Sanità, Rome, Italy

The small protein Bv8 (isolated from amphibian skin), the mammalian Prokineticin 1 (PK1 or EG-VEGF) and Prokineticin 2 (PK2 or mammalian-Bv8) make up a new family of chemokines, involved in many biological activities. These chemokines activate two G-protein coupled receptors, prokineticin receptor 1 (PKR1), mainly distribuited in the peripheral nervous system and prokineticin receptor 2 (PKR2), highly expressed throughout the brain. In rodents, exogenous administration of Bv8 reduces the nociceptive threshold to thermal and mechanical stimuli acting on PKRs on primary sensitive nerves and in spinal cord.
Here we performed a pharmacological characterization of two PKR antagonists, PC1 and PC25, in vitro and in vivo.
In vitro we evaluated the affinity of PC1 and PC25 for PKRs through Bv8-induced activation of G-protein coupled PKRs using BRET (Bioluminescence Resonance Energy Transfer) assay.
In vivo we analyzed the ability of these new compounds to antagonize Bv8-induced thermal hyperalgesia (Paw immersion test, 48°C) and tactile allodynia (Von Frey test) in wilde type (WT) and in PKR1- or PKR2- knockout (KO) mice.

Results. In BRET assay *PC1 shows IC_{50}= 144 nM for PKR1 and IC_{50}= 2964 nM for PKR2, so it is* 20 times more selective for PKR1 than for PKR2.
PC25 has IC_{50}= 8 nM for PKR1 and IC_{50}= 2161 nM for PKR2, so it is ~300 times more selective for PKR1 than for PKR2. Moreover, it shows an affinity for PKR1 ~17 times higher than PC1.
In vivo, we have already demonstrated that thermal hyperalgesia is mediated mainly by PKR1 and tactile allodynia mainly by PKR2. In WT mice, intraplantar (i.pl.) injection of 50 fmol Bv8 induces thermal hyperalgesia and tactile allodynia.
Both thermal hyperalgesia and tactile allodynia are antagonized by the same dose of PC1, 14 pmol i.pl., showing similar affinity for PKRs. PC25 antagonizes thermal hyperalgesia at a dose of 1,4 pmol i.pl., whereas antagonizes tactile allodynia at a dose 3 times higher, ~ 4 pmol i.pl. confirming its higher affinity for PKR1.
In PKR-KO mice a dose of 500 fmol Bv8 is necessary to produce thermal hyperlgesia comparable to that obtained with 50 fmol Bv8 in WT mice.
In PKR1-KO mice, Bv8-induced thermal hyperalgesia is antagonized by i.pl. injection of 150 pmol PC1 and 14 pmol PC25 whereas in PKR2-KO mice hyperalgesia is antagonized by 15 pmol PC1 and 0.04 pmol PC25.
In conclusion, in vitro and in vivo data confirm major selectivity of PC25 for PKR1, according with its major antihyperalgesic efficacy.