Involvement of Nav1.7 voltage-gated sodium channels in the analgesic in vivo activity of some newly-synthesized sumatriptan analogues

R. Carbonara[1], A. Carocci[2], C. Franchini[2], G. Lentini[2], D. Conte Camerino[1], J.-F. Desaphy[1]

[1]Section of Pharmacology and [2]Section of Medicinal Chemistry, Dept. of Pharmacy & Drug Sciences, University of Bari – Aldo Moro, Italy

Perception and transmission of pain in mammals is modulated by Nav1.7 voltage-gated sodium channels expressed in peripheral neurons. Mutations in Nav1.7 cause several neurological disorders, often associated to chronic pain. Treatment of chronic pain is based on the administration of drugs able to exert, through a primary or secondary mechanisms of action, a blockade of voltage-gated sodium channels (VGSCs)[1]. In this study we investigated about the molecular mechanism of action of some sumatriptan derivatives, namely 20b, (R)-31b, and (S)-22b, demonstrated to exert analgesic activity in vivo in a rat model of acute pain[2]. All these compounds are agonists for $5HT1_D$ and/or $5HT1_B$ serotonin receptors[2]. Because of their structural analogy with mexiletine, we tested their ability to block sodium currents using whole-cell patch-clamp experiments in HEK293 cell line permanently transfected with hNav1.7. The stimulation protocol consisted in holding cells at -120 mV and in eliciting sodium currents by depolarizing the membrane at -30 mV, in absence and in presence of the exploratory compounds. We determined tonic and phasic blocks by stimulating cells at 0.1 Hz and 10 Hz frequencies, respectively. Concentration-response curves were drawn at both stimulation frequencies and fitted with the equation: $I_{DRUG} / I_{CTRL} = 1 / (1 + ([Drug] / [IC_{50}]^{nh})$, where IC_{50} was the concentration needed to produce a 50% reduction of sodium currents and nh the slope factor.

Sumatriptan showed a very weak affinity with an IC_{50} of 2319 ± 18 μM at 0.1 Hz and lack of use-dependence. In contrast, the three tested compounds showed a greater affinity to hNav1.7, compared to both sumatriptan and mexiletine (IC_{50} values at 0.1 Hz: 338 ± 18 μM for mexiletine; 132 ± 23 μM for 20b, 118 ± 14 μM for (*R*)-31b; 77 ± 12 μM for (*S*)-22b). They also use-dependently blocked sodium currents (IC_{50} values at 10 Hz: 114 ± 19 μM for mexiletine; 56 ± 7 μM for 20b, 39 ± 4 μM for (*R*)-31b and 23 ± 2 μM for (*S*)-22b). We also investigated the binding site of sumatriptan analogues on VGSCs. Since many sodium channel blockers interact with the channel at level of the binding site of local anaesthetics, involving Phe1586 in skeletal muscle hNav1.4, we tested the most potent (S)-22b on WT and mutated (F1586C) hNav1.4, permanently transfected in HEK293 cells. Results showed a 4-fold lower affinity for F1586C mutant compared to WT, as well as a 3.5-fold lower use-dependent profile, suggesting that (S)-22b may bind with high affinity to the local anaesthetic receptor.

Previous in vivo and binding affinity studies[2] combined with our present results, allowed us to hypothesize that Nav1.7 blockade at high frequencies exerted by (S)-22b and (R)-31b is the main mechanism of action responsible of relief from pain in vivo, while $5HT_{1D}$ serotoninergic agonism may also contribute to (R)-31b analgesic activity. Furthermore, the poor analgesic activity showed by sumatriptan could be explained with a mechanism of action that only involves 5HT receptors. Thus, on the basis on these results, we propose that Nav1.7 blockade is probably more important than the agonism on $5HT_{1B/D}$ receptors to determine a clinically-relevant analgesia and propose (R)-31b and (S)-22b as interesting starting compounds for the synthesis of new analgesic agents with activity on Nav1.7 VGSCs, associated or not with serotonergic agonism.

1. Dib-Hajj SD, Black JA, Waxman SG. Voltage-gated sodium channels: therapeutic targets for pain. Pain Med 2009;10:1260-9.

2. Carocci A, Lentini G, Catalano A, Cavalluzzi MM, Bruno C, Muraglia M, Colabufo NA, Galeotti N, Corbo F, Matucci R, Ghelardini C, Franchini C. Chiral aryloxyalkylamines: Selective 5-HT1B/1D activation and analgesic activity. Chem Med Chem 2010;5(5):696-704.