Pyrazolone derivatives selectively inhibit TRPA1 channel in vitro

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The pyrazolone (Pyr) derivatives (PDs), antipyrine (AntiP), dipyrone (Dip) and propyphenazone (PPh) have been successfully used for more than a century by hundreds of millions of people worldwide in a series of painful diseases, including migraine, colic and post-surgical and neuropathic pain. Several painkillers, such as non-steroidal anti-inflammatory drugs or coxibs, relieve pain by inhibiting COX-1 and COX-2 respectively (Simmons et al., 2004). COX inhibition by PDs is, however, weak, resulting in poor anti-inflammatory effects, which do not match their potent analgesic action, so the exact mechanism of action remains unknown. The transient receptor potential ankyrin 1 (TRPA1) channel is co-expressed by a subset of primary sensory neurons with the capsaicin-sensitive TRP vanilloid 1 (TRPV1), TRPV4 and other TRP channels (Andrade et al., 2012). TRPA1 is activated by an unprecedented series of reactive compounds, that targeting TRPA1 elicit pain and neurogenic inflammation. Such activators include both exogenous pungent irritants, such as mustard oil (AITC), and endogenous pro-algesic agents like reactive/electrophilic by-products of oxidative stress (H2O2, 4-hydroxynonenal and acrolein), which activate TRPA1 via a Michael addition or oxidation reactions of specific amino acid residues (Macpherson et al., 2007). Emerging evidence indicates that TRPA1 contributes to different inflammatory and neuropathic pain (McNamara, 2007). We hypothesized that PDs, selectively inhibit TRPA1 expressed in nociceptors and via this mechanism produce analgesia. Through calcium imaging and electrophysiology we demonstrated that PDs selectively target the TRPA1 channel, inhibiting calcium responses and currents in both transfected and constitutive TRPA1-expressing cells (human embryonic lung fibroblasts and rodent dorsal root ganglia primary neurons). The inhibitory effect (IC50s) on TRPA1 stimulation in human and rodent TRPA1-expressing cells were similar for the two systemically used PDs, PPh and Dip (~60 μM) and higher for AntiP (~600 μM). Moreover, PDs reduced both calcium and electrophysiological responses evoked by electrophilic or reactive TRPA1 selective agonists, such as AITC, acrolein or H2O2, with no effect against the non-reactive agonists, ZnCl2 and icilin, which act independently from binding key cysteine residues of TRPA1 (McKemy et al., 2002). In addition, in a mutated channel, which is lacking of key cysteine and lysine residues (Hinman et al., 2006), calcium responses to non-reactive stimuli, were reduced by the selective TRPA1 antagonist, HC-030031 but unaffected by Pyr and PDs. Mutagenesis studies and in silico analysis, strengthens the proposal that PDs target TRPA1 by binding to cysteine residues required for TRPA1 channel activation by reactive endogenous agonists. Last, their metabolites 4-methylaminoantipyrine, dm-propyphenazone and edaravone, did not antagonize TRPA1 or scavenge TRPA1 reactive agonists in all tests. In conclusion, present findings demonstrate for the first time that PDs, a highly effective group of analgesics used worldwide for more than a century, are selective antagonist of TRPA1 channel evoked by exogenous or endogenous reactive/electrophilic agonists. In addition, the TRPA1-antagonistic profile of PDs is not shared by their metabolites. Thus, present data suggest that the analgesic properties of PDs are mainly ascribed to the TRPA1-blockade.

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