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MEETING ABSTRACT

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K_V7 channels as targets for paracetamol

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Background: Voltage-gated K_V7 channels are expressed in a variety of excitable tissues such as the nervous or cardiovascular system. Neuronal K_V7 channels regulate excitability in neurons; consequently, reduced activity leads to states of hyperexcitability, like epileptic seizures or neuropathic pain. Both pathologies can be treated by increasing currents through these channels, for example via cysteine modification. Despite being used for over 60 years, the mechanism of action of paracetamol remains debated. Metabolites of paracetamol are suggested to mediate its analgesic effect. Most notably, the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) was suggested to also mediate the therapeutic effect via cysteine modification of ion channels.

Methods: Currents through native neuronal and heterologously expressed K_V7 channels were recorded in the perforated voltage-clamp mode. Action potentials in primary first-order (dorsal root ganglion, DRG) and second-order (spinal dorsal horn, SDH) neurons of the pain pathway were recorded in the perforated current-clamp mode. Covalent NAPQI adducts were analyzed by mass-spectrometry analysis. K_V7 channel involvement *in vivo* was tested via inducing inflammation by s.c. injection of carrageenan (100 µl of 2% w/v) into the right hind paw of Sprague-Dawley rats. Nocifensive behavior was tested both with mechanical (using von-Frey filaments) and thermal (Hargreaves test) pain paradigms.

Results: Paracetamol administration (300 mg/kg; i.p.) increased withdrawal forces but not thermal withdrawal latencies. The latter of which could be prevented by co-administration of XE 991 (3 mg/kg, i.p.), a blocker of K_V7 channels. Application of NAPQI (1 µM) reduced the number of action potentials in primary cultures of rat DRG and SDH neurons. This effect could be prevented by application of the K_V7 channel blocker linopirdine (30 µM). NAPQI increased currents through K_V7 channels in both types of neurons but currents remained unaltered in presence of the parent compound paracetamol (100 µM). The current increase could be reproduced in heterologously expressed $K_V7.2$ channels. Application of NAPQI covalently modifies one out of a stretch of three cysteine residues in the S2–S3 linker region (C150–C152). Each cysteine residue can be modified, but only a single cysteine residue is modified at the same time. Alanine substitution of all three cysteine residues prevented K_V7 currents from increasing in response to NAPQI application. In addition, co-transfection of apo-calmodulin also prevented K_V7 currents from increasing in response to NAPQI application.

Discussion: Administration of paracetamol reduces mechanical hyperalgesia in rats but not thermal hyperalgesia. The former effect involves K_V7 channels. On a cellular level, this effect corresponds to reduced action-potential frequencies in first- and second-order neurons of the pain pathway in response to NAPQI application, which, again, involves K_V7 channels. Currents through K_V7 channels are increased in response to the paracetamol metabolite NAPQI, but neither to the parent compound nor to other metabolites. On a molecular level, this effect is mediated by covalent modification of one out of three subsequent cysteine residues in the S2–S3 linker region. This region is known to mediate Ca^{2+}-dependent modulation of K_V7 channels via calmodulin. Indeed, the ability of calmodulin to sense Ca^{2+} seems to be involved in this process.

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