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MEETING ABSTRACT

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K_V7 channels: potential targets for the antinociceptive action of paracetamol

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Background: Paracetamol/acetaminophen (APAP) is a widely used analgesic whose mechanism of action remains controversial. The postulated mechanisms include inhibition of cyclooxygenase enzymes, effects on the descending serotonergic pathways, and involvement of the endocannabinoid system through its metabolite AM404. A small fraction of paracetamol is converted into a reactive intermediate, NAPQI (*N*-acetyl-*p*-benzoquinone imine) by cytochrome P450 enzymes. The M current is characteristic of the neuronal subtypes of voltage-gated potassium channels (K_V7 family). Inhibition of M currents enhances neuronal excitability, while their augmentation causes neuronal silencing with established translational use in pain management and epilepsy. Therefore, effects of APAP and its metabolites on K_V7 channels were investigated.
Methods: tsA201 cells were transfected using plasmids coding for human K_V7 channels. Dorsal root ganglia (DRG) were dissected from 10-day-old rats and dorsal horn (DH) cultures were prepared from newborn rats. They were cultured at 37 °C / 5% CO_2 for 2 days and 21 days, respectively. Electrophysiological recordings were made using the perforated patch-clamp technique.
Results: NAPQI enhanced currents through recombinant homomeric $K_V7.2$ and $K_V7.5$ channels up to 250% and 400% of control, respectively, and inhibited currents through $K_V7.3$ homomers down to 40% of control; both effects were irreversible and concentration-dependent. With $K_V7.2/7.3$ and $K_V7.3/7.5$ heteromers, currents were enhanced to 120% and 250% of control, respectively, in a concentration-dependent manner up to 3 µM NAPQI and depressed at higher concentrations, the effect being irreversible. The tail current in DH and capsaicin-sensitive DRG neurons showed an enhancement up to 250% and 120% of control with 3 µM NAPQI, respectively. There was a significant decrease in the excitability of DRG neurons with 10 µM NAPQI. On application of 3 µM NAPQI for 10 minutes, currents through wild-type $K_V7.2$ homomers exhibited a biphasic enhancement with a plateau at 3 minutes and a further time-dependent which reached equilibrium at 7 minutes. In a single-point cysteine mutant of $K_V7.2$ (C106A), the first phase of current enhancement with 3 µM NAPQI was abolished. The triple cysteine mutant, $K_V7.2$ (CCC150–152AAA) mutant showed a biphasic inhibition with a residual current of 20% at the end of a 10-minute period. In the other single cysteine mutants, $K_V7.2$ (C169A) and $K_V7.2$ (C492A), currents were enhanced by 3 µM NAPQI akin to wild-type channels. The observed effects were irreversible. Paracetamol (1 mM) and AM404 (10 µM) had no effect on homomeric $K_V7.2$ and $K_V7.3$ currents.
Discussion: These results indicate that the analgesic action of paracetamol may involve an enhancement of K_V7 currents by NAPQI as an active metabolite.

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