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P2Y₁ receptors are linked to K_{Ca}2 channels in PC12 cells

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Background

P2Y₁ receptors are widely expressed in the brain, but their signalling mechanisms in neurons remained largely unknown. In sympathetic neurons, recombinant P2Y₁ receptors inhibit voltage-gated Ca²⁺ currents (I_{Ca}) and M-type K⁺ currents.

Methods

Patch-clamp recordings were performed in PC12 cell cultures, P2Y receptor ligands and signaling interceptors were applied.

Results

In PC12 cells stably expressing rat P2Y₁ receptors (PC12-P2Y₁), but not in wild type PC12 cells (PC12-wt), ADP induced rises in intracellular Ca²⁺ with half-maximal effects at 15 ± 1.3 μM. In whole-cell patch-clamp recordings, ADP inhibited I_{Ca} of PC12-P2Y₁ cells (EC₅₀: 6.3 ± 1.7 μM) and of PC12-wt (EC₅₀: 3.8 ± 1.3 μM); this effect was not altered by the P2Y₁ antagonist MRS 2216 (1 μM), but abolished by P2Y₁₂ antagonists. In perforated-patch recordings, ADP inhibited I_M relaxation amplitudes of PC12-P2Y₁ cells with half-maximal effects at 2.0 ± 1.8 μM, but in PC12-wt no such effect was observed. In PC12-P2Y₁, but not in PC12-wt cells, ADP (1–100 μM) caused transient increases in outward currents determined at –30 mV in the perforated-patch, but not the whole-cell mode. ADP-induced currents had reversal potentials between –80 and –90 mV which was close to the calculated K⁺ equilibrium potential (–89 mV). Replacement of 100 mM extracellular Na⁺ by K⁺ shifted the reversal potential of ADP-induced currents to about –10 mV which was again close to the K⁺ equilibrium potential (–17 mV). ADP-induced currents were prevented by thapsigargin (1 μM) and by the phospholipase C inhibitor U73122 (3 μM), but not by an inactive analogue. Finally, the ADP-induced currents were significantly reduced by 100 nM apamin.

Conclusions

These results reveal channels of the K_{Ca}2 family as novel targets for P2Y₁ receptor signalling.

Acknowledgements

Supported by FWF.