Protein kinase A inhibits cell growth induced by overexpression of IK channels

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Intermediate-conductance (IK) potassium channels have been shown to play a key role in the proliferation of different cell types. Blockers of IK channels are effective in inhibiting the growth of lymphocytes, proliferative smooth muscle cells and various cancer cells. We have shown previously that the IK channel is regulated by cAMP-dependent protein kinase (PKA). As the PKA pathway is generally thought to be growth inhibitory, we wished to examine whether PKA can modulate the influence of IK channel activity on cell growth.

An IK-expressing stable cell line was generated in HEK293 cells. The V5 antibody epitope was engineered into the rat IK cDNA, which was transfected into HEK293 cells and cultured in the presence of G418. Western blotting of cell extracts revealed an anti-V5 immunoreactive band at 43kDa which was not present in untransfected HEK293 cells. This band corresponds to the recombinant IK channel subunit. For proliferation assays, cells were seeded at 50,000 cells per 35mm diameter dish and cultured up to 5 days in DMEM containing 10% foetal bovine serum. Cells were counted in duplicate using a haemocytometer.

The IK-expressing stable cell line proliferated at a significantly faster rate compared to the untransfected control HEK293 cells. This enhanced cell growth was completely inhibited by the IK channel antagonist, clotrimazole (10μM). To determine the effect of PKA, cells were exposed to the adenylate cyclase activator forskolin (10μM) during the rapid growth phase. Forskolin prevented the enhanced growth of IK channel-overexpressing cells but had no effect on proliferation of untransfected HEK293 cells.

Serine 332 on the IK channel is a strong PKA consensus site (Neylon *et al.*, 2004). To determine whether the inhibition of cell growth by PKA was due to direct phosphorylation of S332, we generated a stable cell line overexpressing IK channels containing the S332A mutation. Cells overexpressing the S332A mutant grew at a similar rate to those overexpressing wild type IK channels. However, the inhibition of cell proliferation by forskolin was attenuated. Forskolin produced only 30% inhibition of the enhanced growth of cells overexpressing S332A-IK channels, compared to 100% for wild type IK channels.

We conclude that PKA can prevent the influence of IK channels on cell growth, and this effect is mediated partially through direct phosphorylation of S332 on the IK channel.

Neylon, C.B., D'Souza, T., & Reinhart, P.H. (2004) *Pflügers Archiv* **448**, 613-620.