THE EFFECTS OF ACETAZOLAMIDE ON CALCIUM ACTIVATED K^+ CHANNELS IN SKELETAL MUSCLE ARE MEDIATED BY INTERACTION WITH THE ALPHA SUBUNIT

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Acetazolamide (ACTZ) is the prototype of the thiazide derivative used in the treatment of different disorders such as epilepsy, episodic ataxia, glaucoma and neuromuscular disorders. In particular, ACTZ is the most common medications in hypokalaemic periodic paralysis (hypoPP), a neuromuscular disorder characterized by episodes of flaccid paralysis and muscle weakness accompanied by lowering of the serum K^+ concentration. Most hypoPP patients benefit by empiric treatment with ACTZ. Previous studies in our laboratories showed that in the K-depleted rats, the animal model of hypoPP, ACTZ ameliorate the symptoms of hypoPP rats through direct activation of the muscular Ca^{2+}-activated K^+ channels (BK) (1). BK channels play an important role in regulating the excitability of many tissues including skeletal muscle. They are activated by both intracellular calcium and membrane depolarization so they represent an important link to couple the effects of calcium and membrane potential. Structurally, BK channels exist as a tetramer composed of four pore-forming α-subunits either or in association with tissue-specific accessory β subunits. While only one gene encoded for the α-subunit, four putative β-subunits types have been cloned from different gene. The association of the pore-forming α-subunit with different β-subunits in native tissues modifies the pharmacological and biophysical properties of the assembled channels. Since no accessory β subunits have been found in skeletal muscle it's feasible to predict that ACTZ effects on BK channels in this tissue are mediated by interaction with the α subunit alone. To test this hypothesis, HEK293 cells were transiently transfected with expression vectors (pcDNA3) encoding the human α subunit (*hslo*) of BK channels and patch clamp experiments in whole cell configuration were performed to investigate ACTZ effects. BK channels were identified by their voltage and Ca^{2+} dependence and by a sustained currents that do not inactive. Our experiments showed that the external application of 10 μM and 50 μM concentrations of ACTZ to the cell, at negative membrane potentials, enhanced the BK currents by 3 and 6 folds, respectively. At positive membrane potentials, the effects of ACTZ were less pronounced. These results indicate that ACTZ activates *hslo*-BK channels expressed in HEK293 cells and the α subunit is the minimal requirement for activation of BK channels by ACTZ. However, our data do not exclude the possibility that the β subunits may affect ACTZ actions.

1. Tricarico D., Barbieri M., Conte Camerino D. (2000) Ann Neurol.;48(3):304-12.