Biochemical, morphological, and pharmacological evidence for Kv7.4 channels expression in neuronal mitochondria

I. Vinciguerra[1], M. V. Soldovieri[1], G. Paventi[1], P. Ambrosino[1], V. Calderone[2], S. Passarella[1], and M. Taglialatela[1,3]

[1]Dept. of Medicine and Health Science, University of Molise, Campobasso (Italy)
[2]Dept. of Pharmacy, University of Pisa, Pisa (Italy)
[3]Dept. of Neuroscience, University of Naples 'Federico II', Naples (Italy)

Potassium channels in the inner mitochondrial membrane (mitoK channels) participate in the ischemic preconditioning phenomenon, a term referring to the cellular protection against anoxia/ischemia produced by exposure to short, sub-lethal hypoxic insults. In neurons, among the two best studied mitoK channels are mitoK_{ATP} and mitoBK channels, which are regulated in an inhibitory and excitatory fashion, respectively, by nucleotides and divalent cations. The Kv7 family of voltage-gated potassium channels is an additional gene family which includes five members (Kv7.1-Kv7.5), each showing a specific cellular distribution and functional role; mutations in four of the five Kv7 genes result in human channelopathies (**1**). Since Kv7 channels have been shown to play a relevant role in in vitro (**2**; **3**) and in vivo (**4**) models of neuroprotection against anoxic/ischemic events, the aim of the present study has been to investigate their possible presence in neuronal mitochondria,

To this aim, expression of transcripts and proteins for each Kv7 family member was investigated by RT-PCR and western-blotting, respectively, in F11 cells, a hybrid cell line obtained by the fusion of rat embryonic dorsal root ganglion cells with mouse neuroblastoma cells. Western blotting experiments were also performed in subcellular fractions from F11 cells, including mitochondria. Finally, mitochondrial membrane potential ($\Delta\Psi$) was monitored in isolated mitochondria by the safranin O fluorimetric method; a decrease in the safranin O fluorescence (l_{ex}:520 nm, l_{em}:580 nm) indicates an increase in $\Delta\Psi$ (**5**).

RT-PCR experiments revealed that the mRNAs encoding for all Kv7 subunits were expressed in F11 cells; hovewer, Western-blotting experiments using subunit-specific antibodies only detected the expression of Kv7.4 subunits. Subcellular fractionation experiments showed that Kv7.4 subunits are localized in F11 mitochondria, a result also confirmed by immunocytochemistry experiments showing a strong overlap between the distribution of the signals of anti-Kv7.4 antibodies and of the mitochondrial marker mitotracker. $\Delta\Psi$ monitoring experiments revealed that externally added K^+ decreased $\Delta\Psi$ with saturable kinetics, consistent with channel mediated transport. The functional role played by K_{ATP} channels was confirmed by the observation that the mitoK_{ATP} blocker glybenclamide (20 μM) decreased the rate of mitochondrial K^+ transport; noteworthy, kinetic analysis of mitochondrial K^+ transport in the presence and absence of glybenclamide revealed the presence of at least two K^+ transport mechanisms. To investigate the possible contribution of Kv7 channels to mitochondrial K^+ transport, the effects of Kv7 activators (retigabine) or inhibitors (XE991) on 20 mM KCl-induced mitochondrial depolarization were also studied. The results obtained revealed that the rate of 20 mM KCl-induced mitochondrial depolarization was reduced by XE-991 (20 μM) and enhanced by retigabine (10 μM). More importantly, the stimulatory effect of retigabine was fully reversed by XE-991, while it was unaffected by glybeclamide exposure.

Altogether, these results demonstrate that neuronal mitochondria contain Kv7.4 channel subunits which play a critical role in mitochondrial K^+ transport. Future experiments will assess whether the novel mechanisms herein described might be implicated in neuroprotective mechanisms triggered by preconditioning.

REFERENCES

1.Soldovieri et al., Physiology 2011. 2.Boscia et al., Neuropharmacology 2006. 3.Barrese et al., J Cereb Blood Flow Metab 2015. 4.Bierbower et al. J Neurosci 2015. 5.Pastore et al., J Biol Chem 1999.