

KCNQ CHANNELS REGULATE DEPOLARIZATION-INDUCED CHANGES IN INTRACELLULAR Ca^{2+} CONCENTRATIONS IN RAT CEREBROCORTICAL NERVE ENDINGS

¹Rosa Luisi, ¹Elisabetta Panza, ¹Agnese Secondo, ²Maria Martire, ¹Lucio Annunziato, and ^{1,3}Maurizio Tagliatela

¹Div. of Pharmacology, Dept. of Neuroscience, School of Medicine, Univ. "Federico II", Naples; ²Institute of Pharmacology, School of Medicine, Catholic Univ. of Sacred Heart, Rome; ³Dept. of Health Sciences, Univ. of Molise, Campobasso, Italy

Heteromeric assembly of KCNQ2 and KCNQ3 subunits underlie I_{KM} , a neuron-specific voltage-dependent K^+ current that plays a pivotal role in neuronal excitability control by limiting repetitive firing and causing spike-frequency adaptation. Most studies have defined the role of I_{KM} in synaptic integration at the somatodendritic level; however, recent pharmacological and morphological studies have suggested that I_{KM} may also play a relevant presynaptic role. In particular, compounds interfering with I_{KM} can influence depolarization-induced release of neurotransmitters from isolated nerve terminals of various brain regions; while I_{KM} activators reduce, I_{KM} blockers facilitate neurotransmitter release (1,2). Since neurotransmitter release evoked by presynaptic plasmamembrane depolarization is primarily a calcium (Ca^{2+})-dependent phenomenon, the aim of the present study was to investigate the possible involvement of KCNQ channels in the control of the changes in the intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$) elicited by depolarization in rat cortical synaptosomes by means of video-imaging techniques. Synaptosomes from the cerebral cortex of adult male Wistar rats were purified on discontinuous Percoll gradients, layered onto glass coverslips, and loaded with a non-ratiometric fluorescent calcium indicator (Fluo-4 AM; excitation wavelength: 490 nm; emission wavelength: 510 nm). Cerebrocortical synaptosomes were depolarized by two subsequent exposures to an extracellular solution containing 20 mM $[\text{K}^+]_e$ each lasting 30 sec, separated by a 10-min resting period. In control synaptosomes, the two 20 mM $[\text{K}^+]_e$ stimuli elicited comparable increases in Fluo-4 fluorescence intensity ($S_2/S_1 = 0.9 \pm 0.05$; $n \approx 300$). By contrast, when the specific I_{KM} activator retigabine (0.1-30 μM) was perfused onto the synaptosomes for 8 min before the second high $[\text{K}^+]_e$ pulse, a dose-dependent and reversible inhibition of the Fluo-4 fluorescence intensity increase elicited by 20 mM $[\text{K}^+]_e$ was observed ($E_{\text{max}} = 38.2 \pm 7.0\%$; $\text{IC}_{50} = 0.89 \pm 0.16 \mu\text{M}$). Interestingly, XE-991 (20 μM), a specific I_{KM} inhibitor, failed to modify the K^+ -evoked changes in Fluo-4 fluorescence intensity, but it completely abolished the inhibitory action of retigabine (10 μM). Collectively, these data reveal that I_{KM} regulates depolarization-induced $[\text{Ca}^{2+}]_i$ changes in nerve terminals, and suggest that I_{KM} openers, by hyperpolarizing the synaptosomal plasmamembrane, may reduce Ca^{2+} influx through presynaptic voltage-gated Ca^{2+} channels, thus limiting depolarization-induced neurotransmitter release.

References

1. Martire M., Castaldo P., D'Amico M., Preziosi P., Annunziato L. and Tagliatela M. (2004) *J. Neurosci.* 24 (3): 592-597.
2. Martire M., D'Amico M., Panza E., Miceli F., Viggiano D., Lavergata F., Iannotti F., Barrese V., Preziosi P., Annunziato L. and Tagliatela M. (2007) *J. Neurochem.* (*in press*).

