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

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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 neurotrasmitter relase (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 ($[Ca^{2+}]_i$) 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 $[K^+]_e$ each lasting 30 sec, separated by a 10-min resting period. In control synaptosomes, the two 20 mM $[K^+]_e$ stimuli elicited comparable increases in Fluo-4 fluorescence intensity (S_2/S_1 = 0.9±0.05; n ≈ 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 $[K^+]_e$ pulse, a dose-dependent and reversible inhibition of the Fluo-4 fluorescence intensity increase elicited by 20 mM $[K^+]_e$ was observed (E_{max}=38.2±7.0%; IC_{50}=0.89±0.16 µM). 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 $[Ca^{2+}]_i$ 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

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