MULTIPLE MECHANISMS OF TRANSMITTER RELEASE EVOKED BY ELEVATED EXTRACELLULAR K^+: INVOLVEMENT OF TRANSPORTER REVERSAL AND MITOCHONDRIAL CALCIUM

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Different mechanisms can allow translocation of neurotransmitters through the plasma membrane of neuronal terminals. Under physiological conditions, neurotransmitter release occurs by vesicular exocytosis which includes terminal membrane depolarization and gating of VSCC. Evidence exists that vesicular exocytosis can also take place independently of VSCC activation, triggered by Ca^{2+} mobilized from intraterminal stores. Finally, neurotransmitters can exit from nerve terminals through reversal of the transporter (carrier-mediated release). The different mechanisms of neurotransmitter release may coexist. However, it has not been clearly established whether and under what conditions this can occur. In this work we studied the mechanisms underlying the release of GABA from rat cerebrocortex synaptosomes following depolarization with varying concentrations (9-50 mM) of KCl.

[^{3}H]GABA overflow was detectable already at 9 mM and reached a plateau at 50 mM KCl. The selective GABA-uptake inhibitor SKF100330-A did not affect the 9 mM KCl-evoked [^{3}H]GABA overflow, but it decreased that evoked by 15, 35 and 50 mM KCl. The inhibition was directly correlated to the strength of the stimulus applied. Omission of extracellular Ca^{2+} reduced [^{3}H]GABA overflow evoked by 9 mM KCl almost totally and that evoked by 50 mM KCl by about 35%. When SKF100330-A was applied in Ca^{2+}-free medium, the 15 mM KCl-evoked overflow was abolishes but still a portion remained (about 40%) with 50 mM KCl. The external Ca^{2+}-independent [^{3}H]GABA overflow by 50 mM KCl (in the presence of SKF100330-A) was further reduced by entrapped BAPTA, supporting a role of internal calcium. The overflow of [^{3}H]GABA was diminished by dantrolene, blocker of the ryanodine-sensitive endoplasmic reticulum Ca^{2+}-channels, by thapsighargin, inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase, or less so by entrapped heparin, blocker of the IP_3-sensitive Ca^{2+}-channels. The mitochondria Na^+/Ca^{2+} exchanger inhibitor CGP37157 diminished [^{3}H]GABA overflow to a similar extent of BAPTA. In line results were obtained when accumulation of Ca^{2+} into mitochondria was hindered by the Ca^{2+} uniporter inhibitor Ru360 or the mithocondrial ATPase inhibitor olygomicin. KCl (50 mM) raised the intraterminal free Ca^{2+} concentration in synaptosomes loaded with FURA-2 AM also in the absence of extraterminal Ca^{2+} and this effect was counteracted by CGP 37157 and thapsigargin. Vesicular exocytosis, measured by the fluorescent probe acridine orange, by 50 mM KCl in external Ca^{2+}-free conditions was also diminished by CGP 37157 and thapsigargin.

We can conclude that at least three mechanisms support the KCl-evoked [^{3}H]GABA release, depending on the strength of the stimulus applied: i. carrier mediated release; ii. exocytosis involving extracellular Ca^{2+}; iii. exocytosis triggered by mobilization of Ca^{2+} from internal stores. It could be speculated that Na^+ entering during depolarization exchanges Ca^{2+} from mitochondria which, in turn, mobilize Ca^{2+} from the endoplasmic reticulum, a process which appears adequate to sustain exocytosis.

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