Mechanisms of GABA release from cerebellar nerve terminals: role of Na^+/Ca^{2+} exchangers, GAT1 transporter reversal, Ca^{2+}-induced Ca^{2+} release and anion channels

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The aim of the present work was to study some mechanisms of GABA release from mouse cerebellar nerve terminals following stimuli that can be considered 'physiological' and stimuli generated by ion dysregulations typical of some neuropathological conditions.

Glycine $GlyT_2$ transporters are localized on glycine-storing nerve endings. Their main function is to accumulate glycine to replenish synaptic vesicles. Glycine was reported to be costored with γ-aminobutyric acid (GABA) in cerebellar interneurons. It was previously found that GABA uptake elicited glycine release from cerebellar nerve endings by various mechanisms. We now investigated whether and through which mechanisms activation of glycine $GlyT_2$ transporters could evoke release of GABA. Purified mouse cerebellar synaptosomes were prelabeled with [^{3}H]GABA and exposed to glycine in superfusion. Glycine stimulated [^{3}H]GABA release in a concentration-dependent manner. The effect of glycine was insensitive to strychnine or to 5,7-dichlorokynurenate but it was abolished by selective $GlyT_2$ transporters blockade. About 20% of the evoked release was dependent on external Ca^{2+} entered by reversal of plasmalemmal Na^+/Ca^{2+}exchangers. A significant portion of the $GlyT_2$-mediated [^{3}H]GABA release occurred by reversal of GABA GAT1 transporters. Na^+ ions, reaching the cytosol during $GlyT_2$-mediated glycine uptake, likely activated mitochondrial Na^+/Ca^{2+} exchangers, causing an increase in cytosolic Ca^{2+}, which in turn triggered a Ca^{2+}-induced Ca^{2+} release process at inositoltrisphosphate receptors. Finally, the increased availability of Ca^{2+} in the cytosol allowed the opening of anion channels permeable to GABA. Thus, the function of $GlyT_2$ transporters is not only to take up glycine and replenish synaptic vesicles but also to mediate GABA release. Part of the glycine-induced GABA release mechanisms exhibit a significant connection with functional plasmalemmal and mitochondrial Na^+/Ca^{2+} exchangers.

In the second part of the study, GABA release provoked by ion dysregulations typical of some neuropathological conditions such as ischemia was analyzed using purified cerebellar synaptosomes pre-labeled with [^{3}H]GABA and exposed in superfusion to high KCl or veratridine. The overflows caused by relatively low concentrations of the two releasers were almost totally external Ca^{2+}-dependent. Higher concentrations of KCl or veratridine involved also external Ca^{2+}-independent mechanisms. The GABA overflows evoked by veratridine and, less so, the overflow evoked by high KCl, occurred in part by reversal of the GAT1 transporter. The two depolarizing agents did not activate store-operated or transient receptor potential or L-type Ca^{2+} channels. Significant portions of the external Ca^{2+}-dependent overflows evoked by the two releasers involved reversal of plasmalemmal Na^+/Ca^{2+} exchangers. The overflows evoked by high KCl or veratridine were also dependent on Ca^{2+} originated through mitochondrial Na^+/Ca^{2+} exchangers. Ca^{2+}-induced Ca^{2+} release mediated by inositoltrisphosphate receptors participated exclusively in the GABA release stimulated by high KCl which also occurred in a modest portion through anion channels.