Glycerotoxin stimulates exocytosis and endocytosis by increasing intracellular Ca^{2+} in N-type calcium channels expressing cells

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We recently purified a novel neurotoxin from *Glycera convoluta* named Glycerotoxin (GLTx), capable of stimulating neurotransmitter release from N-type Ca^{2+} channels expressing neurons for up to 24h (Schenning *et al.*, 2006). Here, we have found that GLTx also stimulates compensatory endocytosis of synaptic vesicles using styryl dyes and electron microscopy. Furthermore, we have adapted a fluorescent-based assay to monitor intracellular Ca^{2+} flux from both rat brain synaptosomes and human embryonic kidney (HEK) cells over-expressing N, L, P/Q and R-type Ca^{2+} channels. GLTx triggers Ca^{2+} influx in HEK cells expressing rat or human N-type Ca^{2+} channels without affecting cells transfected with L, P/Q or R-type Ca^{2+} channels. In addition, GLTx promoted Ca^{2+} influx in rat brain synaptosomes and an increase in endogenous glutamate released with an EC50 of 50 pM. GLTx is therefore a unique tool available to unravel the mechanism controlling Ca^{2+}-regulated exocytosis and compensatory endocytosis *via* the specific activation of N-type Ca^{2+} channels. Importantly, GLTx was found to act on both rat and human clones of N-type Ca^{2+} channels. GLTx or derivatives could therefore be useful in future human therapy strategies aiming at enhancing neurotransmitter release by selectively stimulating N-type Ca^{2+} channel-expressing neurons.

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