N-type calcium channels contribute to acetylcholine release from parasympathetic but not sympathetic preganglionic neurons in female mice

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Neurons communicate with each other almost exclusively through chemical synapses *via* a mechanism that is dependent on calcium. The link between calcium entry into the nerve terminal and neurotransmitter release has been the subject of considerable study over recent decades. Whilst many of the underlying principles common to all synapses have been elucidated, it is becoming increasingly apparent that the relationship between calcium entry and synapse function varies considerably between synapses. Of particular note is differential expression of calcium channel subtypes which provide the source of calcium necessary for neurotransmitter release. The reasons for such diversity are unclear. Perhaps they allow the vastly different patterns of communication which we see in different neural circuits? As in the central nervous system, peripheral synapses which control autonomic targets show heterogeneity in the calcium channel subtypes they express. Outflow from any particular autonomic pathway is the culmination of activity from a number of nuclei in the brainstem, hypothalamus and higher areas. In turn these activate preganglionic neurons within the spinal cord. Preganglionic neurons in certain pathways, especially those which control reproductive tissues, also participate in reflex circuits regulated at the level of the spinal cord. Preganglionic outflow is relayed to peripheral effectors through synapses in autonomic ganglia. It is now clear that the autonomic pathways controlling different effectors are independently regulated. The fact that each autonomic pathway and hence ganglionic synapse has characteristic patterns of activation suggests that transmitter release may be optimally "tuned" to the requirements of the end organ. In a previous study we demonstrated that in paracervical ganglia of female guinea-pigs acetylcholine (ACh) release from the terminals of sacral preganglionic neurons requires calcium entry from both N-type calcium channels and an undetermined calcium channel, possibly R-type. In contrast, N-type calcium channels play no role in acetylcholine release from the terminals of lumbar preganglionic neurons in the same ganglion (Jobling *et al.*, 2004). One difference between N-type calcium channels and other calcium channel subtypes is that N-type calcium channels are modulated to a greater extent by G-protein coupled receptors. Perhaps the selective involvement of N-type calcium channels in sacral pathways confers greater synaptic plasticity? To determine if our result represents a general mammalian phenomenon we have now investigated the functional role of calcium channels in transmitter release from parasympathetic and sympathetic terminals in paracervical and prevertebral ganglia of female mice. Pelvic or celiac ganglia were taken from mice (3-5 weeks of age) killed under deep anaesthesia following intraperitoneal injection of sodium pentobarbitone (150 mg/Kg). These procedures were approved by the Animal Welfare Committee of the University of Newcastle in accordance with the National Health and Medical Research Council Australian code of practice for the care and use of animals for scientific purposes. Ganglia were pinned in a recording chamber and superfused with HEPES buffered salt solution. Intracellular recordings were made with microelectrodes filled with 0.5 M KCl. Pelvic nerves to paracervical ganglia or splanchnic nerves to celiac ganglia were stimulated via suction electrodes. Preganglionic nerve stimulation evoked suprathreshold EPSPs in the majority of postganglionic neurons. In order to better resolve pharmacological effects of calcium channel antagonists, hexamethonium (30-100μM) was routinely used to reduce the amplitude of EPSPs below threshold. Single electrode voltage-clamp was used to measure EPSC amplitude in the absence and presence of selective calcium channel antagonists. In paracervical ganglia omega conotoxin GVIA, a selective N-type calcium channel antagonist, reduced the amplitude of EPSCs evoked by pelvic nerve stimulation by ($46 \pm 5\%$, $n = 8$), $p < 0.05$). In contrast, in the celiac ganglion, omega conotoxin GVIA had no effect on the amplitude of EPSCs evoked by splanchnic nerve stimulation ($P = 0.2$, $n = 7$). EPSCs in both paracervical and celiac ganglia were resistant to the P-type calcium channel antagonist agatoxin (50 nM, $n = 5$ paracervical, $n = 5$ celiac) and the R-type calcium channel antagonist SNX482 (100 nM, $n = 4$ paracervical, $n = 4$ celiac). These results indicate that in the female mouse, release of ACh from sacral parasympathetic preganglionic neurons requires calcium entry from both N-type and toxin-resistant calcium channels. Release of ACh in sympathetic pathways to prevertebral ganglia does not require calcium entry from N-type calcium channels. The expression of N-type calcium channels in sacral preganglionic neurons may provide a mechanism for selective modulation of these pathways.

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