Cholinergic modulation of pyramidal neuron excitability in the prefrontal cortex

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Muscarinic acetylcholine receptors (mAChRs) are important for normal cognitive function. We investigated the effects of acetylcholine on layer 5 pyramidal neurons in slices of prefrontal cortex obtained from anaesthetized and decapitated 3-6 week-old rats. Bath-application of the mAChR agonist carbachol (10 μM) depolarised neurons by 5.7 ± 1.8 mV (n = 13). In contrast, phasic application of ACh (100 μM; 5 – 1000 ms) resulted in transient membrane hyperpolarisation that inhibited action potential generation. Hyperpolarising responses had a mean amplitude of –6.4 ± 0.8 mV, an onset latency of 295 ± 34 ms, and a duration of 1.35 ± 0.12 s. ACh-induced hyperpolarisations were blocked by atropine (1 μM; n = 7) and the M1 antagonist pirenzepine (1 μM; n = 8), but not by the M2 antagonist methoctramine (1 μM; n = 8), indicating action via M1-type mAChRs. ACh-induced hyperpolarisations were associated with a 25 ± 5% increase in membrane conductance (n = 6) and reversed near the potassium equilibrium potential (-95.7 ± 1.7 mV, n = 6). The response was blocked by intracellular calcium chelation with BAPTA (10 mM; n = 10). Calcium imaging experiments revealed intracellular calcium increases associated with the hyperpolarisations (n = 5). Ryanodine (20 μM) blocked a significant proportion of the response (n = 6, df = 5, $p < 0.05$), implicating calcium release from intracellular stores. The inhibitory action of ACh was unaffected by bath applied TTX (500 nM, n = 4) or antagonists to GABA receptors (100 μM picrotoxin, 1μM CGP55845, and 10 μM SR95531, n = 6). Hyperpolarising responses were apamin sensitive (100 nM), suggesting the involvement of SK-type calcium activated potassium channels (n = 7, df = 6, $p < 0.05$). Importantly, phasic ACh application produced inhibition even during tonic depolarisation with bath-applied carbachol (5 μM, n = 4 of 4 neurons; 7 μM, n = 3 of 4; 10 μM, n = 9 of 14). Our data demonstrate that transient ACh application inhibits prefrontal pyramidal neurons via M1-type receptor-mediated calcium release from intracellular stores and subsequent SK channel activation.