Leptin-induced hypothalamic excitability is mediated by a TRPC-Cav3 complex

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Leptin-induced depolarization in POMC neurons is mediated via a Jak2-PI3 kinase-PLCγ pathway that ultimately activates TRPC channel activity, most likely a TRPC1-TRPC5 heteromer. This TRPC complex-induced depolarization and intracellular calcium release are postulated to trigger action potentials (AP), increasing neuronal excitability. However, the downstream elements of this cascade are not well defined yet. Here we used cultured neurons to establish the role of T-type Ca2+ channels in the leptin signaling pathway in neuron excitability. Hypothalamic cultures were studied from 8-10 days in vitro (DIV). Immunocytochemistry analysis showed that POMC and NPY neurons were present in the culture, with POMC neurons being the majority of the cells analyzed (85 vs. 15%, n=182; $P< 0.05$, Z-test). Electrophysiological experiments confirmed 86% of all neurons tested were Leptin-activated, with their resting potential slightly depolarized, their rheobase decreased and the number of action potentials (APs) increased upon application of 100 nM Lep. Interestingly, leptin application did not directly alter low-voltage activated currents, yet inhibition of T-type channels using 10 μM of NNC 55-0396 completely abolished the effect of leptin, similarly to the effect seen with 100 μM of the TRPC channel blocker 2-APB. Basal excitability was also prevented by the T-type channel blocker, as seen with post-inhibitory rebound experiments and ramp protocols. Evidence shows ion channels work in coordination as part of macromolecular complexes within cells, thus we tested whether TRPC1/5 channels could be detected in complex with CaV3 channels. IP experiments showed both TRPC1 and C5 co-precipitate with either CaV3.1 or CaV3.2 (and vice versa). Moreover, given that the Na/Ca permeability through TRPC1/5 channels is ~0.95, to test if this channel complex is physiologically relevant in the leptin cascade, we assessed the effect of leptin in the presence of intracellular calcium buffers with similar affinities but different binding rate constants. Indeed, the fast chelator BAPTA precluded the local effect of leptin, whereas EGTA (>100X slower buffer) did not alter the leptin response, corroborating that calcium influx through TRPC1/5 and its contribution to membrane depolarization recruit adjacent T-type channels within the complex, increasing neuronal excitability.