IN VITRO NEURONAL DIFFERENTIATION OF EMBRYONIC AND PROGENITOR CELLS DEPENDS ON KININ-B2 RECEPTOR ACTIVITY

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Variations in intracellular calcium concentration $[Ca^{2+}]_i$ controlled by the activity of metabotropic and ionotropic receptors is crucial for neuronal function as well as for the differentiation of stem or progenitor cells into neurons. We have used the murine embryonal carcimoma P19 cell line as an in *vitro model* for studying the function of metabotropic kinin-B2 receptors (B2R) during early neuronal development. Following induction to differentiation, the cells express neuron-specific proteins, reaching highest levels after 7-9 days. At this stage P19 neurons develop synapses and express functional muscarinic and nicotinic acetylcholine receptors. B2R mRNA transcription and protein expression were modulated during differentiation of P19 cells. Functional B2Rs were not expressed in embryonic P19 cells, but following induction to differentiation, P19 cells responded to bradykinin application with an increase in $[Ca^{2+}]_i$. Increasing secretion of bradykinin into the cell culture medium during neuronal differentiation suggests the presence of an autocrine loop between bradykinin and its receptor. Bradykinin secretion was inhibited when P19 cells were differentiated in the presence of the B2R antagonist HOE-140. Moreover, the presence of HOE-140 led to inhibition of the Ca^{2+}-response induced by the acetylcholine receptor agonist carbamoylcholine and decreased gene expression levels of M1-M3 muscarinic receptors on day 8 *(Martins et al. J. Biol. Chem. 280, 19576, 2005)*. Inhibition of the B2R activity during differentiation of embryonic rat neurospheres also resulted in the loss of cholinergic and purinergic receptor-induced calcium responses, pointing at a crucial function of the B2R in cell differentiation to neurons.

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