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Expression of connexin(Cx)26, 32, 43, and 45 in developing rat ventral mesencephalic dopaminergic neurons *in vivo* and their regulation by fibroblast growth factor-2 (FGF-2) and glial cell line-derived neurotrophic factor (GDNF) *in vitro*

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During development neuronal cells follow distinct differentiation patterns, the regulation of which is not fully clarified by now. Developing neurons are coupled by gap junctions, which seem to be important for neuronal differentiation. During ontogenesis, neuronal expression of gap junction connexins and functional coupling reaches a maximum perinatally and afterwards is steadily downregulated with relatively little coupling being left in the adult. A group of substances being well known to regulate differentiation and survival of CNS neurons are protein growth factors such as fibroblast growth factor-2 (FGF-2) and glial cell line-derived derived neurotrophic factor (GDNF). Both have been shown to promote differentiation and survival of developing mesencephalic dopaminergic neurons *in vivo* and *in vitro*. The questions emerging from this constellation were firstly, whether gap junctions are regulated by these growth factors and secondly, if regulation of neuronal gap junctions plays a role in the neuroprotective actions of FGF-2 and GDNF. To answer these questions, different approaches were employed. First, gap junctional communication in developing rat ventral mesencephalon was analyzed *in vivo*. For this purpose, I studied mRNA and proteins of Cx26, Cx32, Cx43 and Cx45 in rat ventral mesencephalon at embryonic stages E12, E14, E16, E18, neonatal P0, postnatal age P4, P7 and in the adult by RT-semiquantitative PCR and immunocytochemistry. It was found that all four gap junction subtypes were differentially regulated during ventral mesencephalic development with their expression patterns correlating to critical steps in their development, such as neurogenesis, radial glia-directed migration, axonal growth and pathfinding, synaptogenesis, as well as differentiation of astrocytes and oligodendrocytes. In a second approach, the effects of FGF-2 and GDNF on expression of Cx26, Cx32, Cx43, and Cx45 in rat E14 ventral mesencephalic neuronal cultures *in vitro* were investigated by RT-semiquantitative PCR, Western blotting and dye transfer studies. I observed that all four connexin proteins were expressed in dopaminergic neurons *in vitro* as identified by immunohistochemical double staining for the dopaminergic neuronal marker tyrosine hydroxylase (TH). FGF-2 significantly upregulated Cx43 mRNA, immunoreactivity and functional coupling, whereas GDNF had no such effect. To test whether the upregulation of gap junctional communication by FGF-2 was involved in mediating the neuroprotective effects of this growth factor, we combined treatment of E14 ventral mesencephalic neuronal cultures with 10ng/ml FGF-2 or GDNF to various concentrations of the gap junction blocking agents,

18α-glycyrrhetinic acid (AGA), carbenoxolone (CBN), and oleamide (OLE). It turned out that gap junction uncoupling agents either alone or in combination with growth factors differentially affected survival of dopaminergic neurons as revealed by counting TH^+ cells. Oleamide, the most specific of the three substances, enhanced survival of dopaminergic neurons in the absence of growth factors and additionally was able to abolish the survival promoting effect of FGF-2. There was no correlation of these effects with neither cell proliferation as detected by BrdU-incorporation nor to cell apoptosis as assayed by TUNEL-staining. To assess the role of gap junction coupling for dopaminergic neuronal loss in a model of Parkinsonism, expression of the four connexins in the midbrain floor of mice treated with MPTP was investigated. In control animals connexin plaques of all four subtypes could be detected in TH+ neurons, whereas in MPTP treated mice, remaining dopaminergic neurons revealed almost no connexin plaques at their cell membranes. Taken together, this study demonstrates the importance of gap junctional communication in mediating or modulating effects of growth factors on dopaminergic neuronal differentiation and survival during development and under pathological conditions. Both, growth factors and gap junctional coupling might closely interact in the control of critical events during ventral mesencephalic differentiation. The differential and restrictive developmental downregulation of connexins during ventral mesencephalic dopaminergic neuronal development *in vivo* seems may be related to growth factor actions seen *in vitro*, especially those of FGF-2 on Cx43 expression *in vitro*. Lastly, the observed mechanisms could be of importance for a better understanding of midbrain development and perhaps even treatment of neurodegenerative diseases such as Parkinsonism.

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