Therapeutic potential of non-neuronal cells in Amyotrophic Lateral Sclerosis (ALS)  
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Introduction
Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the death of motoneurons in the motor cortex, brain stem and spinal cord. The G93A transgenic mouse model expresses a mutant form of human superoxide dismutase (SOD1) and develops motoneuron disease similar to ALS (Rosen, Siddiqui, 1993). It has previously been shown that surrounding non-neuronal cells (glia) have an influence on the survival of motoneurons (Clement et al., 2003; Nagai et al., 2007). We are therefore currently studying the interaction of neuronal and non-neuronal cells in vitro using a co-culture system. In this system, we have addressed the potential neuroprotective capacities of Glucagon-like peptide-1 (GLP-1). GLP-1 has initially been studied as a treatment for type II diabetes based on its major function as insulin secretagogue (John et al., 2008). Neuroprotective capacities of GLP-1 are supposed to arise from a direct effect on cell membrane as well as an effect on local synaptic circuits (Wan et al., 2007).

Methods
Primary Culture
The isolation and in vitro cultivation of embryonic primary motoneurons from individual mouse embryos (gestational age E14/15) were performed by a p75NTR-antibody-based immunopanning technique. Highly enriched motoneuron preparations were seeded on glass coverslips either pre-incubated with poly-L-lysine (diluted 1:1000; Sigma) for co-culture or pre-incubated with laminin (2.5µg/ml; Invitrogen) for monoculture.

Toxicity Experiments
Motoneuron-rich cell fractions (MN) derived from either wildtype or mutant G93A transgenic ALS mice (WT MN/G93A MN) were seeded on laminin or on glial feeder layers of wildtype or transgenic neonatal mouse astrocytes (WT A/G93A A) at an average density of 2.0x10^5 cells/cm^2. After 7 days in vitro (DIV 7), cell cultures were incubated for 24h with 300µM KA. Furthermore, 300µM KA was applied, together with 100nM GLP-1, for 24h, to test for its possible neuroprotective effect.

Immunocytochemistry
Cells were fixed with 4% para-formaldehyde (PFA). Glial feeder layers were stained with antibodies against gial fibrillary acidic protein (GFAP) (1:50; Sigma, Germany) to identify astrocytes. Motoneurons were stained with an antibody against SMI-32 (1:100; Covance, USA) which is specific for motoneurons derived from embryonic spinal cord. The nuclei of all cultured cells were stained with 4',6-diamidino-2-phenylindole (DAPI; Sigma). Cell counts were done in 5 visual fields of each coverslip in a total of 5-7 different preparations for each condition. Counting was performed using an ocular counting grid to quantify all cells on a horizontal stripe crossing the complete well, using a fluorescence microscope (BX-70).

Literature

Results

Figure 1
Reduction of SMI32 positive motoneurons following KA exposure. Increase in cell survival due to GLP-1 co-incubation. Motoneurons were stained using antibodies against SM1-32 (red). Astrocytes were immunopositive for GFAP (green). Stained nuclei of cultured cells appear in blue (DAPI).

Figure 2
GLP-1 protected motoneurons from toxicity induced by KA. G93A motoneurons in co-culture with G93A astrocytes were significantly more sensitive to KA-toxicity than wildtype motoneurons in co-culture with either wildtype or transgenic astrocytes.

Conclusion
Under KA exposure, there was a significantly increased sensibility of the G93A transgenic motoneurons compared to the wildtype ones (p<0.05). When comparing the co-culture of G93A MN/WT A and G93A MN/G93A A, even without addition of GLP-1, larger numbers of transgenic motoneurons in G93A MN/WA system than of those cocultured with G93A astrocytes were counted, while this difference was not observed in the wildtype motoneuron co-culture systems. This may indicate a protective effect of wildtype astrocytes on transgenic motoneurons.

Further experiments and the assessment of different neurotoxic and neuroprotective compounds at different concentrations and in different co-culture conditions will be necessary. The motoneuron-astrocyte co-culture system could represent a valuable in vitro screening tool for novel neuroprotective compounds in ALS and provide more detailed insights into neuron-glia interaction in ALS.

The GLP-1 analog used was Acetyl-GLP-1-(7-34)-amide from Curatis Pharma GmbH, Hannover, Germany.

The zebrafish model was kindly provided by Dr. J. Schöler, Max-Planck-Institut für Psychiatrie, Munich. The GLP-1 receptor agonist Acetyl-GLP-1-(7-34)-amide was synthesized by Forschungszentrum Jülich. The GLP-1 receptor antagonist GLP-1 (8-37)-amide was synthesized by Dr. J. Schöler, Max-Planck-Institut für Psychiatrie, Munich.