THE *IN VITRO* ASCORBIC ACID/DEHYDROASCORBIC ACID CYCLE IN STEM CELLS FROM THE MOUSE SUBVENTRICULAR ZONE

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Brain neurons contain high concentration (10 mM) of ascorbic acid (AA), which is supplied through a sodium-dependent transporter (SVCT2) (1). Since neural stem cells have the potential to produce new neurons, we deemed of interest to ascertain as to whether stem cells from the mouse subventricular zone (SVZ) might possess SVCT2 and therefore accumulate AA. We used neurosphere-derived stem cells that were established from the periventricular region of the forebrain ventricles of adult C57BL/6 mice (2). Cell viability was assessed by MTT assay. Intracellular content and extracellular concentrations of AA were quantified by HPLC. Results. AA (100 μM, incubation time 24h) induced a slight (+11%) but significant increase in stem cell proliferation. When AA 100 μM was added to the stem cell culture, intracellular content of AA reached 0.64±0.05 μg/mg protein after 30 min and 2.27±0.25 μg/mg protein after 2h30 min. When AA 100 μM was incubated with fluoretin (200 μM), an inhibitor of AA transporter, intracellular AA content was greatly decreased by about 92% after 30 min and by about 89% after 2h30 min of incubation. In astrocytes, the oxidized form of AA, dehydroascorbic acid (DHAA), is reduced back to AA and released in the extracellular compartment via the glutamate-ascorbate heteroexchange (3). We deemed of interest, therefore, the study of the fate of DHAA added to the stem cell culture. DHAA (200 μM, incubation time 24h) induced a slight (+10%) but significant increase in stem cell proliferation. When DHAA (8 mM) was added to the stem cell culture, DHAA concentrations in the medium progressively decreased, but no intracellular DHAA could be detected. On the contrary, it could be detected AA, which progressively increased both within the cells (up to 98±6.5 ng/mg ptotein) and in the medium (up to 340±32 ng/5 ml medium) after 2h30 min of incubation. Conclusions. The main finding of this study is that stem cells from the mouse SVZ possess SVCT2 which allows the cell to accumulate AA. Moreover, stem cells are able to induce the DHAA reduction back to AA. The latter data is consistent with the ability of stem cell to protect dopamine and L-DOPA from autoxidation.

1 Hediger M.A. Nature Med. 8: 514-517, 2002. **2** Pluchino S., Zanotti L., Rossi B., Brambilla E., Ottoboni L., Salani G., Martinello M., Cattalini A., Bergamini A., Furlan R., Comi G., Constantin G. and Martino G. Nature, 436: 266-271,2005. **3** Miele M., Boutelle M.G. and Fillenz M. Neuroscience 62: 87-91, 1994.