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Ascorbic Acid in the Brain*

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The biochemical function of ascorbic acid

Though the entire biochemical role of ascorbic acid is not fully known, a number of processes requiring ascorbic acid have been described (Murray *et al.* 1990). They include: a) hydroxylation of proline and lysine in the synthesis of collagen, b) degradation of tyrosine (the effect consists in keeping copper in a reduced state, the same applies for *c)* and *f)*, c) synthesis of adrenaline and noradrenaline from tyrosine by dopamine-β-hydroxylase, d) bile acid synthesis, e) participation in various reductive reactions in steroidogenesis (the amount of ascorbic acid in the adrenal cortex shows a marked decrease when steroidogenesis is stimulated), f) stimulation of iron absorption, and g) inhibition of nitrosamine formation during digestion (ascorbic acid acts as a water-soluble antioxidant).

Vitamin C thus forms a redox system in the organism with three components: reduced ascorbic acid, the ascorbate radical (semidehydroascorbic acid) and dehydroascorbic acid. The greater part of this redox system is localized in the cytoplasm. The oxidation of ascorbic acid to dehydroascorbic acid gives rise to the free ascorbate radical, which evidently forms the bulk of free radicals in the plasma (Sasaki *et al.* 1982). For the production of other (more toxic) radicals the interaction of ascorbate anions with copper and iron is necessary. Besides forming free oxygen radicals, ascorbic acid is also able to neutralize them. Many authors attribute the beneficial effect of vitamin C on various pathological processes to this particular effect of ascorbic acid. Moreover, the ascorbic acid is an important detoxicant factor. C avitaminosis reduces biotransformation enzyme activities and the cytochrome P 450 level in the liver and adrenals (Zannoni *et al.* 1982). In guinea-pigs with C avitaminosis, the decrease in the cytochrome P 450 level is accompanied in afflicted animals by an increase of serum and liver ceruloplasmin concentrations (Kábrt *et al.* 1981). Ginter (1986) assumes that ascorbic acid stimulates biotransformation enzymes by producing activated oxygen (free radicals or peroxides), so that the cytochrome P 450 cycle is shortened. C hypovitaminosis is also associated with a significant decrease in microsomal monooxygenase activities.

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The subsequent administration of ascorbic acid leads to a characteristic parabolic reaction; the activity of these enzymes rises to a given maximum, while further administration of vitamin C results in their decrease. The explanation is that vitamin C, in different concentrations, is able to bind as well as to produce free oxygen radicals and thus to protect cell membranes from the effects of peroxides. Murata *et al.* (1985) observed that the inactivation of phages δA, MS_2 and ϕ 6 by ascorbic acid is prevented by scavengers for the superoxide radicals (O_2) and hydroxyl radicals ($OH^{\cdot}$). Catalase fully prevented the phage inactivation whereas superoxide dismutase only partially prevented it. Their results indicate that OH is the major reactive species that is directly responsible for the inactivation of phages by ascorbic acid.

Ascorbic acid in the brain

Ascorbic acid is distributed unevenly in the organism, the most highly saturated tissues being some of the endocrine glands and the CNS. Between these tissues and the plasma there is a high concentration gradient testifying to an oxygen-dependent active transport mechanism (Martin 1961). The existence of an active transport mechanism is borne out by the finding that the intravenous injection of a large amount of ascorbic acid is not followed by an increase of ascorbic acid concentration in the brain and the CSF. Hammerstrom (1966), who administered ^{14}C-labelled ascorbic acid to guinea-pigs and studied the time course of their whole body radioactivity, suggested that ascorbic acid first of all penetrates from the blood into the choroid plexus and from there into the CSF and the brain; he considered the possible existence of a blood-brain barrier for ascorbic acid and assumes that the choroid plexus is evidently the site where ascorbic acid can enter the CSF and then the brain.

The brain ascorbic acid concentration of newborn mammals is high and falls with growth and maturation (Adlard *et al.* 1973, Kratzing *et al.* 1982, Schreiber and Trojan 1990b). In the rat, adult values of ascorbic acid are reached at the age of about six weeks (Loscalzo *et al.* 1980) and are higher in the brain than in any other tissue. Schaus (1957) described a significant decrease in the amount of ascorbic acid in the human cerebral cortex in relation to age, but there were no changes in the muscle ascorbic acid concentration.

The principal functional significance of ascorbic acid in the CNS is its ability to inhibit the peroxidation of membrane phospholipids. Ascorbic acid is considered to be one of the main endogenous factors protecting the brain cell membranes from the action of peroxides. Seregi *et al.* (1978) emphasized that a physiological ascorbic acid concentration in the brain inhibits lipid peroxidations. In addition, ascorbic acid modulates the activity of neurotransmitter systems, causes a reduction of receptor-bound ligands (Tolbert *et al.* 1979) and inhibits isolated Na^+,K^+-ATPase activity (Ng *et al.* 1985). It also increases the amount and the distribution density of acetylcholine receptors (Knaack *et al.* 1986) and inhibits the activity of peroxide, oxygen and hydroxyl radicals (Bodaness and Chan 1979, Wagner *et al.* 1985).

The effect of hypoxia on ascorbic acid in the brain

The high ascorbic acid content in nervous structures and its scavenger effect on free radicals give rise to the question which changes of brain ascorbic acid occur in the most common form of brain damage, i.e. during hypoxia. In particular, it raises the question whether ascorbic acid (by scavenging free radicals) affects any post-hypoxia "reperfusion" toxicity in the brain.

It is known (Johshita *et al.* 1989) that the free radical scavenger ONO-3144 mitigates the consequences of cerebral ischaemia (recirculation-induced oedema and postischaemic hypoperfusion). Other findings also indicate the significance of peroxidation in the brain tissue (Suno and Nagaoka 1989). Arad *et al.* (1985) studied the possible function of ascorbate in hypoxia-induced peroxidation but failed to find any changes in the ascorbic acid content of either the cerebral cortex or the hypothalamus after hypoxia or asphyxia.

In our experiments (Schreiber *et al.* 1989), the effect of acute (30 kPa, 1 day) and chronic hypoxia (41 kPa, 13 x 8 h) on the ascorbic acid content of the liver, adrenals, plasma, cerebrospinal fluid and brain was studied in 18-day-old rats. Acute hypoxia caused a significant drop of ascorbic acid concentration in the adrenals, while plasma and CSF ascorbic acid levels rose significantly compared with the controls. Chronic hypoxia was manifested by a significant increase in ascorbic acid concentration in all examined samples, except for the bulbus olfactorius and vermis cerebelli (Tab. 1).

Table 1
The effect of acute and chronic hypoxia on ascorbic acid content in brain tissue of 18-day-old rats

Tissue	Controls	Acute hypoxia	Chronic hypoxia
Bulbus olfactorius	2.26 ± 0.38 (9)	3.09 ± 0.33 (9)	3.02 ± 0.55 (9)
Cortex	2.23 ± 0.37 (9)	2.97 ± 0.39 (9)	3.98 ± 0.59 (9)*
Hippocampus	2.98 ± 0.35 (9)	2.86 ± 0.36 (9)	4.76 ± 0.74 (9)*
Vermis cerebelli	3.85 ± 0.58 (9)	5.10 ± 0.46 (9)	5.41 ± 0.85 (9)
Medulla oblongata	1.88 ± 0.23 (9)	1.42 ± 0.43 (9)	4.23 ± 0.78 (9)*

* – *significantly different from controls ($p < 0.05$)*

A single i.p. dose of ascorbic acid (1 mg/g body weight) (Schreiber and Trojan 1990a) caused a marked increase of ascorbic acid content in five regions of the brain of 18-day-old rats as compared with the intact controls. Acute hypoxia modified the increase. Conversely, chronic intermittent hypoxia combined with the

administration of ascorbic acid led to a mild, nonsignificant increase of ascorbic acid contents in all examined parts of the brain except the vermis cerebelli in which the increase was significant.

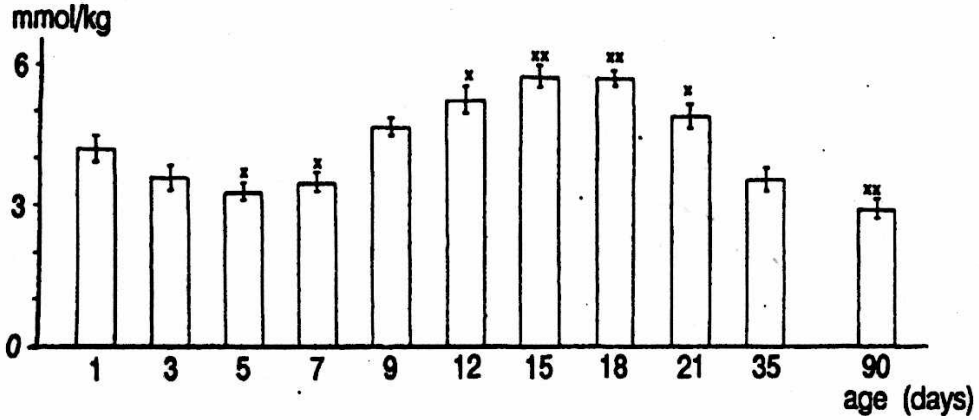

Fig. 1

Developmental changes of ascorbic acid content (mmol/kg w.w.) in vermis cerebelli. x, xx indicate significant differences ($P<0.05$ and $P<0.01$, respectively) as compared to the values found in 1-day-old rats.

In a study on ascorbic acid concentration in five brain regions of rats aged from one to 90 days (Schreiber and Trojan 1990b), we found that the values varied in bulbus olfactorius from 2.9 to 4.5 mmol/kg wet weight, with significant decreases on the 21st, 35th and 90th day of postnatal life. A similar pattern was found in the cerebral cortex, with significant drops on the 21st and 90th day. Variable values were obtained in the hippocampal region, where significant decreases were found on the 5th, 12th, 15th, 21st, 35th and 90th day. The development of ascorbic acid content in vermis cerebelli has a characteristic initial depression that is followed by an increase with the maximum on the 18th day (Fig. 1). Significant decreases were recorded on the 5th, 7th and 90th day of life whereas significant increases were present on the 12th, 15th, 18th and 21st day. The lowest ascorbic acid contents were found in the medulla oblongata, where significant decreases were recorded on the 5th, 21st, 35th and 90th day of life and a significant increase on the 18th day. All significances are related to the values in 1-day-old rats. The changes of ascorbic acid during development in the other brain areas displayed a similar pattern with smaller differences between individual ages. Our results thus show developmental as well as hypoxia-induced changes of ascorbic acid concentration in various parts of the brain.

The findings on the biochemical functions of ascorbic acid are briefly reviewed, with a special reference to its function in the brain. The increase in ascorbic acid concentration in the brain during chronic hypoxia, increased accumulation of administered ascorbic acid in the vermis cerebelli after hypoxia and developmental changes of ascorbic acid concentration in different parts of the brain provide the evidence that ascorbic acid may have a functional significance in the brain, particularly in young rats.

References

ADLARD B.P.F., DE SOUZA S.W., MOON S.: The effect of age, growth retardation and asphyxia on ascorbic acid concentration in developing brain. *J. Neurochem.* **21**: 877–881, 1973.

ARAD I., SIDI A., SHOHAMI E.: Effect of acute hypoxia on ascorbate content of plasma, cerebral cortex and adrenal gland. *J. Neurochem.* **45**: 766–769, 1985.

BODANESS R.S., CHAN P.C.: Ascorbic acid as a scavenger of singlet oxygen. *FEBS Lett.* **105**: 195–196, 1979.

GINTER E.: Vplyv výživy na aktivitu cholesterol-7-alfa-hydroxylázy. *Čs. fyziol.* **35**: 259–270, 1986.

HAMMERSTROM L.: Autoradiographic studies on the distribution of C^{14}-labelled ascorbic acid and dehydroascorbic acid. *Acta Physiol. Scand.* Suppl. 289: 1–70, 1966.

JOHSHITA H., ASANO T., HANAMURA T., TAKAKURA K.: Effect of indomethacin and a free radical scavenger on cerebral blood flow and edema after cerebral artery occlusion in cats. *Stroke* **20**: 788–794, 1989.

KÁBRT J., PŘIBYL T., OČENÁŠKOVÁ J., DOBROVOLNÁ E., KLEIN O.: Akutní skorbut u morčat. Vliv na hladiny ceruloplasminu a cytochromů P-450 a b5. *Čas. lék. čes.* **120**: 211–214, 1981.

KNAACK D., SHEN I., SALPETER M.M. PODLESKI T.R.: Selective effects of ascorbic acid on acetylcholine receptor number and distribution. *J. Cell. Biol.* **102**: 795–802, 1986.

KRATZING C.C., KELLY J.D., OELRICHS B.A.: Ascorbic acid in neural tissues. *J. Neurochem.* **39**: 625–627, 1982.

LOSCALZO B., CRISCI A., PADAVANO N., AGRUSTA M., SCOGNAMIGLIO.T.: *Multidisciplinary approach to brain development.* DIBENEDETTA (ed.), Elsevier, Amsterdam, 1980, pp. 39–44.

MARTIN G.R.: Studies of the tissue distribution of ascorbic acid. *Ann. N.Y. Acad. Sci.* **92**: 141–147, 1961.

MURATA A., SUENAGA H., HIDESHIMA S., TANAKA Y., KATO F.: Hydroxyl radical as the reactive species in the inactivation of phages by ascorbic acid. *Agric. Biol. Chem.* **50**: 1481–1487, 1985.

MURRAY R.K., MAYES P.E., GRANNER D.K., RODWELL V.W.: *Harper's Biochemistry.* Appleton Lange, Norwalk, 1990.

NG Y.C., AKERA T., HAN C.S.: Ascorbic acid: an endogenous inhibitor of isolated Na^+,K^+-ATPase. *Biochem. Pharmacol.* **34**: 2525–2530, 1985.

SASAKI R., KUROKAWA T., TEROKUBOTA S.: Nature of serum ascorbate radical and its quantitative estimation. *Tohoku J. Exp. Med.* **136**: 113–119, 1982.

SEREGI A., SCHAEFER A., KOMPLOS M.: Protective role of brain ascorbic acid content against lipid peroxidation. *Experientia* **34**: 1056–1057, 1978.

SCHAUS R.: The ascorbic acid content of human pituitary, cerebral cortex, heart and skeletal muscle and its relation to age. *Am. J. Clin. Nutr.* **5**: 39–41, 1957.

SCHREIBER M., NOVÝ P., TROJAN S.: Acute and chronic hypoxia influence on ascorbic acid content in various areas of the brain, liver, adrenals and in biological fluids of 18-day-old rats. *Sbor. lék.* **91**: 129–136, 1989 (in Czech).

SCHREIBER M., TROJAN S.: Effect of ascorbic acid administration on its content in cerebral tissue of intact and hypoxic 18-day-old rats. *Sbor. lék.* **92**: 85–88, 1990a (in Czech).

SCHREIBER M., TROJAN S.: Ascorbic acid content in some areas of the rat brain during development. *Sbor. lék.* **92**: 354–369, 1990b (in Czech).

SUNO M., NAGAOKA A.: Inhbition of lipid peroxidation by idebenone in the mitochondria in the presence of succinate. *Arch. Gerontol. Geriatr.* **8**: 291–297, 1989.

TOLBERT L.C., THOMAS T.N., MIDDAUGH L.D., ZEMP J.W.: Effect of ascorbic acid on neurochemical, behavioral and physiological systems mediated by catecholamines. *Life Sci.* **25**: 2189–2195, 1979.

WAGNER G.C., CARELLI R.M., JARVIS M.F.: Pretreatment with ascorbic acid attenuates the neurotoxic effects of methamphetamine in rat. *Res. Commun. Chem. Pathol. Pharmacol.* **47**: 221–228, 1985.

ZANNONI V.G., HOLSZTYNSKA E.J., LAUS S.: Biochemical functions of ascorbic acid in drug metabolism. *Adv. Chem. Ser.* 200: 349–368, 1982.

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