

## The Influence of Hypoxia on the Formation of Amino Acids from Oxidized Substrates in the Rat Brain *in vivo*\*

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### Summary

Hypoxia can influence the development of pathological processes in the brain not only through the decrease of oxidative phosphorylation but also by synthesis and release of excitatory amino acids and GABA. We studied the influence of hypobaric ( $pO_2$  8.6 kPa) and normobaric hypoxia ( $pO_2$  5.27 kPa) on the transformation of  $U\text{-}^{14}C$  glucose and  $2\text{-}^{14}C$  acetate into some amino acids connected with the tricarboxylic cycle. Hypobaric hypoxia and normobaric hypoxia have different effects on metabolic processes in neuronal and glial cells. The formation of the studied amino acids from  $U\text{-}^{14}C$  glucose (neuronal compartment) was more decreased than the formation of amino acids from  $2\text{-}^{14}C$  acetate (glial compartment). This could be a consequence of higher sensitivity of neuronal than glial mitochondria to oxygen deficit or a result of uncoupling of amino acid formation in neurones. An unchanged synthesis of glutamine from acetate in glial cells during hypoxia may result in a protective metabolic effect.

### Key words:

Hypobaric hypoxia – Normobaric hypoxia – Amino acid formation – Neuronal cells – Glial cells

It is generally accepted that the damage of brain cells resulting from reduced oxygen supply during hypoxia or anoxia starts with acidosis, which is mainly the result of glycolysis and leads to the cytotoxic action of excitatory amino acids, mainly glutamic acid (Auer and Siesjö 1988, Kogure *et al.* 1988, Siesjö 1988, Olney *et al.* 1989). It is assumed that, when the oxygen supply is lowered or arrested, a part of protons from neurones is exchanged for sodium by an antiport system. When the ATP level is lowered, the sodium pump is not sufficiently effective and the neuronal membrane is depolarized. Depolarization opens the voltage-dependent  $Ca^{2+}$  gate. The increased  $Ca^{2+}$  in neurones cannot be pumped out, as the  $Ca^{2+}$  pump is not effective. Calcium ions activate a number of enzymes, some of them with

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autodigestive properties. The excitotoxic amino acids are extruded from injured neurones and destroy neurones with excitatory amino acid receptors (Kogure *et al.* 1988). The extent of damage of brain cells depends on the size and duration of oxygen deprivation, on the glycaemia and on the function of adaptation mechanisms.

The oxidative metabolism of the brain, weighing about 2 % of the body but utilizing about 20 % of total body oxygen consumption (Kety 1957), is not only a source of energy for brain activity but, in the course of oxidative metabolism, excitatory amino acids, glutamic and aspartic, are produced in large quantities.

A characteristic property of the brain tissue is the incorporation of a substantial part of carbon atoms of oxidized substrates (60–70 %) into amino acids as shown in experiments *in vivo* and *in vitro*. Glucose is mainly oxidized in neurones and acetate and some amino acids are mainly oxidized in glial cells. Pyruvate and ketone acids formed in the Krebs cycle are intermediates in this process (Balázs and Cremer 1973, Minchin and Beart 1975, Turský *et al.* 1979). Glutamate and aspartate concentrations in the brain are nearly four times higher than those of glucose. A part of glutamate produced in neurones as well as of GABA and probably of aspartate passes out from neurones and is caught by astrocytes. In astrocytes, glutamate is transformed into glutamine directly while aspartate, GABA and acetate are transformed into glutamate and glutamine *via* processes associated with the Krebs cycle. In the glutamine-glutamate cycle, glutamine passes from astrocytes into neurones where it is a precursor of synaptosomal glutamate and GABA (Shank and Aprison 1981, Hertz 1979, Yudkoff *et al.* 1988, Paulsen and Fonnum 1989).

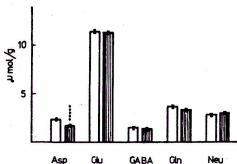
Decreased brain oxidation caused by hypoxia can influence the development of pathological processes not only by reducing oxidative phosphorylation and the release of excitatory amino acids and GABA, but also by affecting the production of these acids. In the initial phase of rat ontogenesis, the transformation of oxidized substrates into amino acids is small and the brain tissue is less sensitive to the lack of oxygen (Patel and Balász 1975).

We studied the influence of normo- and hypobaric hypoxia on the transformation of U-<sup>14</sup>C glucose and 2-<sup>14</sup>C acetate into amino acids. Male rats weighing 350–400 g were used. Radioactive precursors (2 MBq in Tris-Krebs medium containing (mM): NaCl 127; KCl 4.7; CaCl<sub>2</sub> 2.5; MgSO<sub>4</sub> 1.2; KH<sub>2</sub>PO<sub>4</sub> 1.2; Tris 30; glucose 5, pH adjusted to 7.4) were injected i.p. 10 min before the end of two-hour hypoxia. Rats were decapitated and their heads were frozen in liquid nitrogen. The brains were pulverized and about 100 mg brain pulver was homogenized in 2.5 % TCA. Amino acids were separated from substrates and acid metabolites on a cation exchanges and their total radioactivity was measured. After electrophoretic separation of amino acids their quantity and radioactivity were measured. The procedure was described in detail before, together with the method of isolation and determination of the radioactivity of proteins and lipids (Turský and Laššánová 1977).

### *Hypobaric hypoxia*

The conditions of hypobaric hypoxia corresponding to an altitude of 7 000 m were achieved in a hypobaric chamber. The air pressure was lowered at a rate of

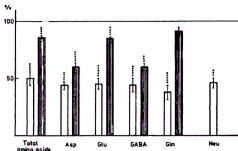
1 000 m altitude difference per 5 s using a table suction pump. The conditions in the chamber were as follows: temperature 24 °C, air pressure 41 kPa, PO<sub>2</sub> 8.6 kPa.



**Fig. 1**

The levels of aspartic acid (Asp), glutamic acid (Glu), GABA, glutamine (Gln) and neutral amino acids (Neu) in the brain of adult rats after hypobaric hypoxia (vertically hatched columns). Controls are represented by open columns. Means of 30 determinations  $\pm$  S.E.M. Significant difference is denoted by five dots ( $p < 0.001$ ) above the respective column.

The hypobaric hypoxia used was relatively mild, but it was near to a fatal oxygen decrease for rats which is achieved at 8 000 m (Trojan 1982). Positive conditioned reflexes were impaired at repeated 7 000 m hypoxia (Mareš *et al.* 1985). In our experiments, only the level of aspartic acid decreased (Fig. 1). Wood *et al.* (1968) found an 18 % increase of GABA levels in experiments at the simulated altitude of 7 300 m, and an increase of 40 % at 9 700 m, while in our experiments GABA levels did not change. Hypobaric hypoxia lasting eight hours at a simulated 7 000 m altitude increased the glutamate and aspartate content and decreased glutamine levels (Koudelová and Trojan 1980).



**Fig. 2**

Effect of hypobaric hypoxia on the incorporation of <sup>14</sup>C from U-<sup>14</sup>C glucose (open columns) and 2-<sup>14</sup>C acetate (vertically hatched columns) into amino acids expressed in % of the controls. Means of 8–12 determinations  $\pm$  S.E.M. Significant differences are denoted above the respective columns: three dots ( $p < 0.01$ ), four dots ( $p < 0.005$ ), five dots ( $p < 0.001$ ).

The incorporation of  $^{14}\text{C}$  from glucose (specific activity 37 MBq/mmol, supplied by ÚVVVR, Prague) into total and individual amino acids decreased during hypobaric hypoxia approximately to 50 % (Fig. 2). The incorporation into glutamine was the most suppressed. On the other hand, the incorporation from acetate (specific activity 1.85 GBq/mmol, supplied by Zentr. Inst. f. Kernforschung, Akad. d. Wissenschaften, Dresden) into amino acids, taking place in glial cells, was only somewhat lowered (Fig. 2). This shows that, in glial cells, the Krebs cycle and the synthesis of associated amino acids are little impaired during hypoxia. The incorporation of  $^{14}\text{C}$  from acetate into glutamine corresponded to more than 90 % of the controls and the relative specific activity of glutamine (relative to glutamate), which is an indicator of the activity of glutamate metabolism in the glial compartment, was higher than in the controls (Fig. 3).

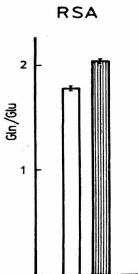


Fig. 3

Effect of hypobaric hypoxia (vertically hatched column) on relative specific activity of glutamine formed from  $^{14}\text{C}$  acetate (RSA = specific activity of glutamine : specific activity of glutamate). Controls are represented by the open column. Means of 8–12 determinations  $\pm$  S.E.M.

The fact that the formation of amino acids in the course of acetate oxidation was not significantly decreased might indicate that the saturation of glial mitochondria with oxygen was preserved. According to Chance and Schindler (1964), the pressure of oxygen in mitochondria is 0.27–0.4 kPa. In *in vitro* experiments, the oxidation in mitochondria was not suppressed at a  $p\text{O}_2$  of 0.066 kPa (Lübbers and Starlinger 1975). Decreased incorporation of  $^{14}\text{C}$  into amino acids in the course of  $^{14}\text{C}$  glucose oxidation in neurones might be due to insufficient saturation of neuronal mitochondria, which should be more sensitive to the oxygen deficit than are glial mitochondria. It is also possible that, under hypoxic conditions, oxidation in neurones might occur without concomitant formation of amino acids, as in newborn rat brains.

The incorporation of  $^{14}\text{C}$  from  $^{14}\text{C}$  glucose and  $^{14}\text{C}$  acetate into proteins and lipids was affected in a similar way as its incorporation into amino acids (Fig. 4).

#### Normobaric hypoxia

A mixture of 95 % nitrogen and 5 % oxygen was passed continuously through the chamber with experimental animals. The oxygen pressure of 5.27 kPa was lower than at hypobaric hypoxia. Some of the experimental rats died during a two-hour periods. Animals weighing 350–400 g were more resistant than those weighing 180–200 g.

The levels of all the studied amino acids changed, with the exception of GABA. Aspartic and glutamic acid decreased to 55 % and to 90 %, respectively. Glutamine and neutral amino acids increased to 118 % and 123 % (Fig. 5). The incorporation of  $^{14}\text{C}$  glucose was strongly inhibited in the neuronal compartment. Only 10–20 % of carbon was incorporated into total amino acids, glutamate, aspartate and GABA as compared with the controls. The incorporation into glutamine and neutral amino acids was less affected; it decreased to 48 % and 67 %, respectively (Fig. 6). The lower levels of glutamate and aspartate corresponded to the lower  $^{14}\text{C}$  incorporation. The increase of glutamine and of neutral amino acids was connected with a substantially less marked decline of  $^{14}\text{C}$  incorporation into these amino acids. Alanine is synthesized from glucose by pyruvate transamination. Conger *et al.* (1981) described an increase of alanine in the ischaemic gerbil brain. Ischaemia lasting for 20 min increased alanine to 285 % and decreased glutamate to 86 %. These authors employed the ratio alanine : glutamate as an index of ischaemic brain damage. We did not measure alanine, but it is possible to deduce from the relation of the incorporation and the levels of neutral amino acids and glutamate that the index alanine : glutamate was increased in our experiments.

Schurr *et al.* (1987) described a protective effect of glutamine on neuronal functions during cerebral hypoxia. They showed the effect of preincubation of hippocampal slices in a medium containing 0.1–5.0 mmol/l glutamine on the renewal

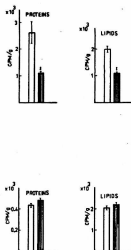


Fig. 4

Effect of hypobaric hypoxia (vertically hatched columns) on the incorporation of  $^{14}\text{C}$  from U- $^{14}\text{C}$  glucose (upper part) and 2- $^{14}\text{C}$  acetate (lower part) into proteins and lipids. Controls are represented by open columns. Means of 9–11 determinations  $\pm$  S.E.M. Significant differences are denoted by two dots ( $p < 0.05$ ) above the respective columns.

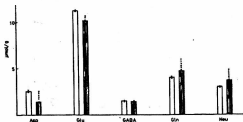
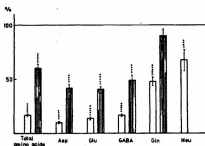


Fig. 5

The level of amino acids in the brain of adult rats after normobaric hypoxia (vertically hatched columns). Controls are represented by open columns. Means of 30 determinations  $\pm$  S.E.M. Significant differences are denoted above the respective columns: two dots ( $p < 0.05$ ), five dots ( $p < 0.001$ ).

of synaptic function after hypoxia. The renewal of synaptic function was achieved in 44 % of cases without preincubation, while it was 90 % with 1 mmol/l glutamine. On the other hand, Goldberg *et al.* (1988) found that glutamine enhanced hypoxic injury in cortical cell cultures. Regardless of the glutamine concentration, the glutamate receptor antagonist 2-amino-5-phosphonovalerate substantially reduced hypoxic injury.

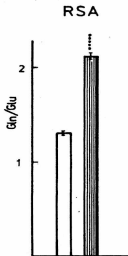


**Fig. 6**

Effect of normobaric hypoxia on the incorporation of <sup>14</sup>C from U-<sup>14</sup>C glucose (open columns) and 2-<sup>14</sup>C acetate (vertically hatched columns) into amino acids expressed in % of the controls. Means of 6–8 determinations  $\pm$  S.E.M. Significant differences are denoted above the respective columns: four dots ( $p < 0.005$ ), five dots ( $p < 0.001$ ).

**Fig. 7**

Effect of normobaric hypoxia (vertically hatched column) on relative specific activity of glutamine formed from <sup>14</sup>C acetate (RSA = specific activity of glutamine : specific activity of glutamate). Control is represented by the open column. Means of 8–12 determinations  $\pm$  S.E.M. Significant difference is denoted by five dots ( $p < 0.001$ ) above the respective column.



We assume that the different effects of glutamine are due to the use of preparations on different levels of tissue organization. In the slices, glutamatergic neurones, which are most sensitive to hypoxia, receive excitatory impulses moderated by GABA interneurones as glutamine is a precursor of both glutamate and GABA in synapses (Paulsen and Fonnum 1989, Lust *et al.* 1988). Folbergrová *et al.* (1989) called attention to the role of equilibrium between excitatory and inhibitory impulses during ischaemia. According to Schurr *et al.* (1987), glutamine might have a protective effect in the brain *in vivo*.

**Fig. 8**

Effect of normobaric hypoxia (vertically hatched columns) on the incorporation of  $^{14}\text{C}$  from U- $^{14}\text{C}$  glucose (upper part) and 2- $^{14}\text{C}$  acetate (lower part) into proteins and lipids. Controls are represented by open columns. Means of 6–8 determinations  $\pm$  S.E.M. Significant differences are denoted above the respective columns: four dots ( $p < 0.005$ ), five dots ( $p < 0.001$ ).

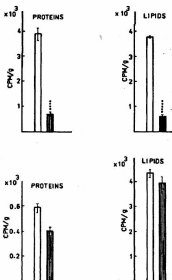
The increased level of glutamine and relative high incorporation of  $^{14}\text{C}$  from glucose into glutamine during hypoxia could be the result of an increased release of glutamate from neurones and its transition into astrocytes where glutamine synthesis occurs.

Carbon incorporation from acetate into amino acids was lowered during normobaric hypoxia as follows: total amino acids to 60 %, glutamate and aspartate to 40 %, and glutamine to 90 %. The formation of glutamate and aspartate was also suppressed in glial cells. This can indicate that the saturation of glial cells with oxygen was insufficient. Under such conditions, the formation of glutamine was only slightly changed and the relative specific activity of glutamine (related to glutamate) was significantly higher than in the controls (Fig. 7).

The incorporation of  $^{14}\text{C}$  from glucose and acetate into proteins and lipids is shown in Fig. 8. The incorporation from glucose was diminished more strongly than that from acetate, similarly as the incorporation into amino acids.

### *Concluding remarks*

The breathing of air containing about 8 % of oxygen (during hypobaric hypoxia) was practically without effect on the amino acid formation from  $^{14}\text{C}$  acetate, connected with the Krebs cycle. At the same time the incorporation of  $^{14}\text{C}$  from glucose was halved. This decrease could be the consequence of a higher sensitivity of neuronal (compared to glial) mitochondria to the oxygen deficit or a result of uncoupling of amino acid formation from the oxidative processes. The breathing of air containing 5 % oxygen (during normobaric hypoxia) lowered the formation of amino acids in neurones more than in glial cells. The synthesis of glutamine (occurring in glial cells) was unchanged. This fact shows that sufficient ATP was available. Adequate production of glutamine during hypoxia can be a protective metabolic reaction. Its effect can depend on the role of glutamine as a precursor of synaptosomal GABA. The decrease of glutamate and aspartate formation from oxidized substrates during hypoxia could represent an adaptive mechanism rather than the effect of strongly suppressed mitochondrial oxidation.



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