Physiol. Res. 41: 363 - 367, 1992

Hypoxia and Fatty Acids in Immature Nervous Tissue in the Rat

J. MOUREK, J. BAŠE, L. ŠMÍDOVÁ

Institute of Physiology, First Faculty of Medicine, Charles University, Prague

Received February 20, 1992
Accepted April 8, 1992

Summary

In experiments on 2-day-old rats (Wistar strain, our own breed), we studied the effect of altitude hypoxia (9000 m, 60 min) on the proportion of individual fatty acids in the brain (the cortex + the diencephalon + the cranial third of the mesencephalon). We found that hypoxia significantly altered the proportion of the various fatty acids, with a significant increase in the proportion of group n-3 polyenoic fatty acids at the expense of saturated and monoenoic acids. The results fully confirm the conception that one of the most important mechanisms responsible for the high resistance of new born mammals to oxygen deficiency is the ability of immature nervous tissue to activate, in particular, elongation (the elongation of fatty acids) and/or lipogenetic processes.

Key words
Fatty acids – Hypoxia – Immature brain

Introduction

This study is a continuation of a series of studies of developmental changes of fatty acids in the rat plasma and brain, including the effect of hypoxia (Mourek *et al.* 1986, Šmídová *et al.* 1986, 1990, Mourek 1989 a,b), and in the plasma of full-term or premature newborns (Mourek *et al.* 1987, Vízek *et al.* 1992, in press).

No explanation has yet been found for the high resistance of newborn mammals to oxygen deficiency, although it is considered to be very important from both the practical and the theoretical aspect and has also been acknowledged for a very long time. Analyses of this phenomenon brought to light important facts – e.g. immature thermoregulation (Adolph 1948), possible better utilization of oxygen (Hahn 1953), lower oxygen consumption by the young organism in an environment poor in oxygen (Mourek 1959), high resistance of respiratory processes in the CNS of newborn rats to lower pH (Mourek and Trojan 1963) and, of course, the significance of anaerobic glycolysis and the genesis of usable energy by this route (Himwich 1951).

In the 1950s, first Raiha (1954) and then Villee (1957) expressed the hypothesis that lipid synthesis, in immature tissue, could be a way of eliminating hydrogen in the presence of oxygen deficiency. Their ideas evoked only a minor response among physiologists and neonatologists, however. Some experiments were carried out on newborn rats in an attempt to find an answer (Villee 1957), but the methodological facilities at that time did not yield unequivocal results.

We studied this problem step by step, on the basis of our own experiments. In 1961 (Mourek *et al.*) we demonstrated that newborn rats, in a nitrogen environment, survived longer after the administration of malonate and later (Hrachovina and Mourek 1978, Hrachovina *et al.* 1983) we reported high NADP-dependent enzyme activities in the brain of newborn and infant rats. This, together with the finding of minimal pH changes in the nervous tissue of newborn rats incubated under anaerobic conditions (compared with older and adult rats), similarly as its ability to regenerate reduced NADP (Mourek 1989a), and findings on the development of CNS mitochondria (i.e. of their membranes and metabolic activities) (Mourek *et al.* 1975, Krasinskaya *et al.* 1985, Dobešová and Mourek 1986) prompted us to carry out further experiments, the results of which are given below.

Material and Methods

Fatty acids (FA) were determined by gas chromatography in the brain tissue of 2-day-old rats (Wistar strain, our own breed). They were isolated and determined by the method described by Svennerholm *et al.* (1978) and modified by Baše (1978). The FA were detected as methyl esters, using a Carlo-Erba 2351 apparatus (for a detailed description see Šmídová *et al.* 1984). The FA are expressed as their percentage proportion in the total amount = 100 %).

Laboratory rats aged two days exposed for 60 min to altitude hypoxia corresponding to 9000 m. The temperature in the low-pressure chamber was maintained at 25 °C. The atmospheric pressure at the given altitude was 30.7 kPA and the pO_2 was 6.4 kPA.

On terminating hypoxia, the rats were killed immediately by decapitation. The brains were rinsed in Ringer solution and were dissected on a cooled block, separating the cerebellum, the medulla oblongata and the caudal part of the mesencephalon from the rest of the brain. We thus used the brain without the cerebellum and the phylogenetically oldest parts. The control measurements were performed similarly, but after the young were removed from the nest they were decapitated without being exposed to hypoxia. The number of measurements in both groups was eight (n=8). In Tab. 1, n stands for the total number of individual FA in the given group - e.g. saturated (8-12), 8:0 (n=8), 10:0 (n=8), 12:0 (n=8), i.e. the sum total of all the measurements was 24.

The results were processed by the cybernetics department of our institute and we wish to thank Mrs Dohnalová for this part of the work.

Table 1

Total changes in the proportion of the individual groups of fatty acids in the brain of 2-day-old rats exposed to hypobaric hypoxia (9000 m, 60 min). n = number of measurements. Arithmetical means (x) ± S.E.M. NS statistically nonsignificant differences. Sigma = sum total.

Fatty acids	n	Control	n	Hypoxic	p
Saturated (8-12)	24	1.81±0.66	24	2.75±0.99	NS
Saturated (14-18)	24	44.82±1.32	24	40.17±0.67	<0.01
Saturated (iso)	32	1.18±0.13	32	1.15±0.15	NS
R Saturated	80	47.81±0.50	80	44.07±0.41	<0.01
Monoenoic F.A.	48	18.28±0.44	48	16.01±0.41	<0.01
Polyenoic FA (n-3)	56	15.36±1.13	56	20.96±1.47	<0.01
Polyenoic FA (n=6)	72	17.28±0.80	72	17.08±0.40	NS
Polyenoic FA (n-9)	24	1.00±0.40	24	1.41±0.53	NS
R Polyenoic	152	33.64±0.56	152	39.45±0.50	<0.01
R Sat/ R Mono		2.5		2.6	
R n-6/R n-3		1.1		0.8	

Results

The results are summarized in Tab. 1. Hypoxia led to a significant decrease in the proportion of saturated FA (in particular of palmitic and stearic acid). The proportion of short and medium-chain saturated FA (C 8:0 to 12:0) did not alter significantly.

Hypoxia caused a significant decrease in the proportion of monoenoic FA (palmitooleic and oleic acid). However, it significantly increased ($p<0.01$) the proportion of type n-3 polyenoic FA /particulary 20:4 (n-3). The proportion of type n-6 polyenoic FA (17 %) was not affected significantly by hypoxia.

The proportion of all polyenoic FA (n-3, n-6 and n-9) in the brain of hypoxic rats rose significantly owing to the increase in type n-3 FA. The index n-6 : n-3 fell as a result of these changes, while the index satur. : monoen. remained unaltered.

Table 2
The first ten most represented fatty acids (FA) in the brain of 2-day-old control rats and of animals exposed to the action of hypoxia. % = the proportion of the given fatty acid in the total amount (= 100 %). + denotes statistically significant differences.

	Control		Hypoxic	
	F.A.	%	F.A.	%
1	16:0	28.3	16:0	25.5$^+$
2	18:0	14.0	18:0	12.7$^+$
3	18:1	13.0	18:1	11:5
4	20:4 n-6	10.6	22:6 n-3	11.0
5	22:6 n-3	9.8	20:4 n-6	10.8
6	16:1	4.7	20:4 n-3	8.0$^+$
7	20:4 n-3	3.7	16:1	4.0$^+$
8	14:0	2.4	8:0	2.3$^+$
9	22:4 n-6	1.6	14:8	1.9
10	8:0	1.0	22:4 n-6	1.5

Tab. 2 shows ten of the most represented FA in the given part of the brain in the order of their proportion. We can see that some unsaturated FA shift higher up the scale in hypoxic rats. It is worth noting that hypoxia did not affect the proportion of arachidonic acid.

Tab. 3 documents all statistically significant changes produced by hypoxia in the representation of individual FA.

Discussion

The increase in the proportion of polyenoic fatty acids (type n-3), such the total increase in the proportion of fatty acids from 33.6 % to 39.4 %, in the brain of hypoxic rats, are two findings which fully concur with our previous data and with a number of supporting findings in the literature, showing that the brain of newborn mammals possesses a mechanism for coping with oxygen deficiency. In our opinion, fatty acids in the immature tissue are a suitable vehicle for the removal of hydrogen. It is known that immature nervous tissue is adequately equipped with an enzymatic apparatus facilitating elongation (Bourre 1980). We ourselves have demonstrated that NADP-dependent enzyme activities, such as glucose-6-phosphate dehydrogenase or isocitrate dehydrogenase, are higly active in the brain of newborn and very young rats (Hrachovina and Mourek 1978, Hrachovina *et al.* 1983). Lastly - as was mentioned in the introduction in earlier studies we already reported findings which support this hypothesis (the ability to regenerate reduced NADP by immature nervous tissue homogenates under anaerobic conditions, accompanied by only minor changes in the pH (Mourek 1989 a). We realize, of course, that the previously found mechanism participating in the defence of the immature organism against oxygen deficiency (a decrease in oxygen consumption, resistance of respiratory processes in the CNS to a decrease in pH, immature thermoregulation and the utilization of ATP from accentuated anaerobic glycolysis) cannot be understimated. Křeček and Šťepánek (1953) found that the high energy carrier content of the brain of newborn rats fell only very slowly during anoxia compared with the rate at which this decrease occurs in the brain of adult animals.

In this situation, i.e. in the presence of oxygen deficiency, an alternative route - the previously postulated "hydrogen-sink effect" and hence a lipogenetic or, more likely, an elongation (and desaturation) route - evidently comes into use. Anyway, both processes are included in the prospective plan of evolution as demonstrated on many occasions (Svennerholm *et al.* 1978, Šmídová *et al.* 1986, Mourek *et al.* 1986, etc). Unsaturated fatty acids actually accumulate in the brain during early ontogenesis (Sinclair 1975). Cook and Spence (1973) demonstrated a high desaturation capacity and dependence of elongation processes on the presence of NADPH (and NADH) and of malonyl-CoA in the immature nervous tissue. Villee and Hegerman (1958) found high lipogenetic activity in liver slices from foetal rats, under both aerobic and anaerobic conditions.

More supporting evidence also exists. Goodridge (1973) demonstrated that lactate (formed in the presence of oxygen deficiency) markedly supported FA synthesis in the immature brain tissue.

We are aware that the FA which we identified have different original localizations and genesis (the neuron glia relations, different types of membranes, etc). Similarly, the proportional expression of the results is only relative.

It is equally conceivable, however, that the relatively lower oxygen supply which accompanies

foetal development – and often the perinatal phase also, as we demonstrated in the rat (Mourek 1976) – could actually represent, precisely because of its nature, the physiological modulator turning at least some metabolic activities in the direction of lipogenetic and elongation processes. An increase in the proportion of long-chain unsaturated fatty acids is regarded as an indication and typical parameter of structural and functional maturation of the CNS.

Table 3
Hypoxia-induced statistically significant differences in the proportion of individual fatty acids (FA) in the brain of 2-day-old rats. Other details as in Tab. 1.

F.A.	n	Control	n	Hypoxic	p
8:0	8	1.06 ± 0.50	8	2.33 ± 0.86	< 0.05
14:0	8	2.37 ± 0.25	8	1.89 ± 0.08	< 0.05
16:0	8	28.36 ± 0.79	8	25.56 ± 0.48	< 0.01
18:0	8	14.08 ± 0.40	8	12.71 ± 0.17	< 0.05
14:0 iso	8	0.17 ± 0.04	8	0.03 ± 0.02	< 0.05
17:0 iso	8	0.21 ± 0.02	8	0.08 ± 0.02	< 0.01
16:1	8	4.72 ± 0.16	8	4.01 ± 0.10	< 0.01
18:1	8	13.06 ± 0.26	8	11.53 ± 0.16	< 0.01
20:4 n-3	8	3.72 ± 1.50	8	8.06 ± 1.0	< 0.05
20:5 n-3	8	0.55 ± 0.33	8	0.25 ± 0.02	< 0.05
22:5 n-3	8	0.70 ± 0.10	8	1.58 ± 0.53	< 0.05
22:6 n-3	8	0.85 ± 0.49	8	11.00 ± 0.56	< 0.05
20:2 n-6	8	0.08 ± 0.02	8	0.30 ± 0.07	< 0.05

References

ADOLPH E.F.: Tolerance to cold and anoxia in infant rats. *Amer. J. Physiol.* **155**: 366 – 377, 1948.

BAŠE J.: A simple preparation of methylesters of fatty acids for gas chromatography estimation (in Czech). *Průmysl potravin* **29**: 339 – 340, 1978.

BOURRE J.M.: Origin of aliphatic chains in brain. In: Symposium No 14 (INSERM) *Neurological Maturation Affecting Myelination*. N. BAUMANN (ed), Elsevier Press, Amsterodam, 1980, pp. 187 – 206.

COOK H., SPENCE M.W.: Biosynthesis of fatty acids in vitro by homogenates of developing brain. Desaturation and chain elongation. *Biochim. Biophys. Acta* **369**: 129 – 141, 1973.

DOBEŠOVÁ Z., MOUREK J.: Development of resistance of the outer membrane of brain and liver mitochondria. *Physiol. Bohemoslov.* **35**: 277 – 280, 1986.

GOODRIDGE A.G.: On the relationship between fatty acid synthesis and the total activities of acetylCoA carboxylase and fatty acid synthetase in the liver of prenatal and early postnatal chick. *J. Biol. Chem.* **448**: 1932 – 1938, 1973.

HAHN P.: Changes in resistance to anoxia during postnatal development in the rat (in Czech). *Čs. Fysiol.* **2**: 299 – 305, 1953.

HIMWICH H.E.: Brain Metabolism and Cerebral Disorders. Williams and Wilkins, Baltimore 1951.

HRACHOVINA V., MOUREK J.: Developmental changes in malate-dehydrogenase and isocitrate dehydrogenase activity in the rat brain (in Czech). *Sb. lék.* **80**: 97 – 104, 1978.

HRACHOVINA V., TROJANOVÁ M., MOUREK J.: The influence of age and starvation on glutamate dehydrogenase and glucoso-phosphatedehydrogenase activity in the rat brain (in Czech). *Sb. lék.* **85**: 201–210, 1983.

KRASINSKAJA I.P., MOUREK J., DRAHOTA Z., DOBEŠOVÁ Z., RAUCHOVÁ H.: Oxidation of acetoacetate and palmitylcarnitine by brain and liver mitochondria from suckling and adult rats. *Physiol. Bohemoslov.* **34**: 121–125, 1985.

KŘEČEK J., ŠTĚPÁNEK F.: Phosphate metabolism in the brain of young rats (in Czech). *Čs. fysiol.* **2**: 287–292, 1953.

MOUREK J.: Oxygen consumption during ontogenesis in rats in environments with high and low oxygen content. *Physiol. Bohemoslov.* **8**: 106–111, 1959.

MOUREK J.: Influence of age and starvation on pO_2 and pCO_2 and haematocrit values in the rat. *Physiol. Bohemoslov.* **25**: 245–250, 1976,

MOUREK J.: The possible importance of fatty acids in the immature brain and the hypoxia state. V. Internat. Berlin Symposium on Infant Mortality. Abstract 1988, p.16

MOUREK J.: PH, pCO_2 and NADP changes during aerobic and anaerobic incubation of the brain cortex of young and adult rats. Physiol. Bohemoslov. **38**: 223–230, 1989a.

MOUREK J.: Hypoxia and the brain (developmental aspects) (In Czech) V. Congress of Czechosl. Neurochemistry, Vysoké Tatry 1989b, Abstract 21.

MOUREK J., JÍLEK L., TROJAN S.: Changes of resistance to nitrogen anoxia and stagnant anoxia after the intraperitoneal administration of Na–malonate and monoiodic acid during ontogenesis in the rat (in Czech). *Čs. Fysiol.* **10**: 268, 1961.

MOUREK J., TROJAN S.: The effect of low pH on oxygen consumption in vitro by rat nervous tissue during postnatal development. *Physiol. Bohemoslov.* **12**: 372–376, 1963.

MOUREK J., PRUŽKOVÁ V., SVOBODOVÁ Z., KRAML J.: Enzymatic activities in mitochondria isolated from the rat brain during development. *Develop. Psychobiol.* **8**: 447–452, 1975.

MOUREK J., BAŠE J., ŠMÍDOVÁ L., KOUDELOVÁ J., KOHOUT M: : Effect of acute hypoxia on fatty acid proportion in the plasma of rats of different ages. *Physiol. Bohemoslov.* **35**: 43–51, 1986.

MOUREK J., BAŠE J., ŠMÍDOVÁ L., MIKOVÁ M., VÍZEK K., MELICHAR V.: Fatty acid values in the plasma of neonates, umbilical cord and maternal blood. *Physiol. Bohemoslov.* **36**: 503–510, 1987.

SVENNERHOLM, L., VANIER M.T., JUNGBJER B.: Changes in fatty acid composition of human brain myelin lipids during maturation. *J. Neurochem.* **30**: 1383–1390, 1978.

SINCLAIR A.J.: Long-chain polyunsaturated fatty acids in the mammalian brain. *Proc. Nutr. Soc.* **34**: 287–291, 1975.

ŠMÍDOVÁ L. BAŠE J., KOUDELOVÁ J., MOUREK J.: Developmental fatty acid changes in different parts of the rat brain. *Physiol. Bohemoslov.* **33**: 427–436, 1984.

ŠMÍDOVÁ L. BAŠE J., MOUREK J., ČECHOVA I.: Proportion of individual fatty acids in the non-esterified (free) fatty acid(EFA) fraction in the serum of laboratory rats of different ages. *Physiol. Bohemoslov.* **39**: 125–134, 1990.

RÄIHÄ C.E.: Tissue Metabolism in the Human Fetus. *Cold Spring Harbor Symp. Quant. Biol.* **19**: p.143, 1954.

VILLEE C.A.: Physiology of the Prematurity. Publ.: The J. Macy Jr. Found., New York 1957.

VILLEE C.A., HEGERMAN D.D.: Effect of oxygen deprivation on metabolism of fetal and adult tissues. *Amer. J. Physiol.* **194**: 457–462, 1958.

VÍZEK K., MOUREK J., BAŠE, J., ŠMÍDOVÁ L., MIKOVÁ M., ČECHOVÁ I.: Fatty acids in the serum in premature human newborns (in Czech). *Sb. lék.* – accepted in press (1992).

Reprint Requests

J. Mourek, Institute of Physiology, First Faculty of Medicine, Charles University, CS-128 00 Prague 2, Albertov 5