INCREASE IN SUCCINATE DEHYDROGENASE ACTIVITY IN MICE BRAIN DURING CHRONIC HYPOXIA

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RESUMEN . Se midió la actividad de la succinato deshidrogenasa (SDH) en siete regiones cerebrales de ratones expuestos a tres semanas de hipoxia hipobárica (450 torr, 4380 metros por encima del nivel del mar), y se comparó con los controles normóxicos de nivel del mar. En el grupo hipóxico se encontró un aumento del 40% en la actividad de la SDH en la corteza y el hipocampo. Éste podría ser un mecanismo compensatorio frente a la disminución de la actividad de otros componentes de la cadena respiratoria en condiciones de hipoxia hipobárica, como una estrategia para mantener la producción normal de ATP.

Palabras claves: Metabolismo cerebral; Succinato deshidrogenasa; Hipoxia; Ratones

RÉSUMÉ: Accroissement de l'activité de la succinate déshydrogénase dans le cerveau des souris au cours de l'hypoxie chronique.

On a mesuré l'activité de la succinate déshydrogénase (SDH) dans sept régions cérébrales de souris exposées à trois semaines d'hypoxie hypobare (450 torr, 4 380 m d'altitude) et on a comparé avec les contrôles normoxiques du niveau de mer. Dans le groupe hypoxique on a constaté une augmentation de 40 % de l'activité de la SDH dans le cortex et dans l'hippocampe. Cette augmentation pourrait être un mécanisme compensateur face à la diminution de l'activité d'autres composants de la chaîne respiratoire dans des conditions d'hypoxie hypobare, comme une stratégie pour maintenir la production normale d'ATP.

Mots-clés : Métabolisme cérébral, Succinate déshydrogénase, Hypoxie, Souris.

SUMMARY: Succinate dehydrogenase (SDH) activity was measured in seven brain regions of mice exposed to three weeks of hypobaric hypoxia (450 torr, 4380 meters above sea level) and compared to to normoxic sea level controls. In the hypoxic group we found an increased activity of SDH in cortex an hippocampus of 40%. These results can be explained as a compensatory mechanism for the decreased activity of other components of the respiratory chain in hypobaric hypoxia, as an strategy to mantain the normal ATP production.

Key Words: Brain metabolism; Succinate dehydrogenase; Hypoxia; Mice

INTRODUCTION

Human brain has one of the highest metabolic rates in the body, while representing just 2% of total body weight. It accounts for 20% of the total body weight oxygen consumption and 95% of its metabolic demands is aerobically accomplished (1,2), so the normal function of the brain is almost entirely dependent on an adequate supply of oxygen for the synthesis of ATP via oxidative energy metabolism..

At high altitudes, where the oxygen arterial PO_2 is reduced almost to half sea level pressure, it would be expected that brain function is affected in some degree. An extreme case is the Chronic Mountain Sickness, which is characterized in part by neurologic and psychiatric symptoms such as headache, paresthesias, depression, physic and mental fatigue (3). Also the risk of migraine and strokes is considerably increased (4).

Changes induced by prolonged exposure to hypoxia in cerebral energy metabolism have been reported (5), such as increase in glucose transport across the blood brain barrier (6) and increased brain glucose consumption (7), reduction in the respiratory activity of mitochondria, including citochrome C oxidase (complex IV) and NADHCo Q reductase activities (Complex I) (8,9). Both enzymes are located in the inner mitochondrial membrane. The succinate dehydrogenase is a component of the Krebs cycle and is also a member of the mitochondrial respiratory chain (complex II) (10).

In order to determine if other enzymes of the mitochondrial respiratory chain are also altered under this metabolic stress, we evaluated the activity of Complex II in mice brains exposed to prolonged hypoxia.

MATERIALS AND METHODS

Litter mates of male Balb/c mice 3 were obtained after weaning at 21 days of age. One group (n=7) was transported to Cerro de Pasco (4380 meters above sea level. A second group remained at sea

level under similar conditions as the control group (n=7).

After three weeks of exposure to hypoxia mice were decapitated and brains quickly removed and stored at -70 oC until use. The following brain regions were dissected: frontal cortex, remainig cortex, cerebellum, hippocampus, hypotalamus, talamus, and corpus striatum. All the procedures were carried out at 4 oC. In brief, tissue samples were homogenized in phosphate buffer 0,3M ph 7,4 (10% w/v), and the SDH activity was assayed spectrophotometrically on 600 nm using Phenazin Methosulfate (PMS) and 2,6 Dichlorofenol indophenol (DCFIF) as final electron acceptor as previously described by Singer (11). Concentrations of proteins were measured by the method of Lowry using bovine serum albumine as the standard (12). The results were expressed as the mean ± SD values and comparison between controls and hypoxic animals were evaluated by Student´s t test (table 1).

Table 1. Succinate dehydrogenase activity (mmol/min/mg protein) in brain regions of mice exposed to three weeks of hypobaric hypoxia.

	Control	**Hypoxic**
Frontal cortex * (n=7)	61,9 ± 12,8	100,7 ± 37,6
Rest of cortex * (n=7)	43,2 ± 10,7	60,7 ± 14.6
Cerebellum (n=7)	68,8 ± 12,8	76.0 ± 22,9
Hippocampus * (n=5)	16,4 ± 2.6	21,2 ± 3,9
Talamus (n=5)	17,5 ± 4.9	20,3 ± 3,9
Hypotalamus (n=7)	19,6 ± 9,3	17,9 ± 9,3
Striatum (n=7)	15,1 ± 6,1	12,7 ± 2,1

Values are means ± SD; n = number of mice.
Significantly different from normoxic controls by Student´s t-test, p<0,05.

RESULTS

After three weeks of exposure to hypoxia mice showed reduced body weight and had higher hematocrits when compared to controls. The mean body weight (g ± SD) was 24,9 ± 1,1 for controls and 19,6 ± 2,1 for hypoxic mice (p<0,005). The hematocrit (% ± SD) was 47,7 ± 3,2 in controls and 60 ± 1,8 in hypoxic mice (p<0,001).

Succinate dehydrogenase activity was increased 40% in the cortex and hippocampus of hypoxic animals when compared with sea level group. The other areas evaluated did not show a significant change (table 1).

DISCUSSION

Prolonged exposure to chronic hypoxia induces changes in brain energetic metabolism as described previously, including a reduction of complex I and complex IV activities (8,9). These changes in the respiratory chain may affect the synthesis of ATP. However Harik et al. (7) have demostrated that ATP levels in brains of rats exposed three weeks to hypobaric hypoxia remain unchanged. This normal ATP levels may be achieved at least in part by an increment in SDH activity as a compensatory mechanism for the maintenance of normal electron flux through the respiratory chain. Previous studies have also demonstrated changes in the SDH activity induced by hypoxia in some brain regions and other tissues (13-16).

As Robin stated, there could be alternative pathways as adaptations to chronic hypoxia in mammals (17). One of the possible involved mechanisms is the increase of succinate thiokinase within the Krebs cycle, produced by the anaerobic metabolism of glutamate as occurs in heart (18,19). The proper knowledge of this pathway in chronic hypoxia warrants further investigation.

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