ADAPTATION OF THE BRAIN'S MICROCIRCULATION TO PROLONGED HYPOBARIC HYPOXIA

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RESUMEN: Adaptación de la Microcirculación Cerebral a la Hipoxia Hipobárica Prolongada

Se revisa algunos de los efectos de la hipoxia hipobáricamoderada (0.5 atmósferas) sobre la microcirculación cerebral en ratas adultas. La vascularización cerebral aumenta luego de hipoxia prolongada, pero el curso en el tiempo de la respuesta adaptativa y su reversibilidad no se conocen. No hemos encontrado un aumento significativo en la vascularización cerebral luego de 4 días de hipoxia, pero sí un incremento significativo de de aproximadamente de 33% luego de 1 semana.La vascularización cerebral continuó aumentando, alcanzando un máximo de aproximadamente 70% sobre los valores controles luego de 2 semanas de hipoxia, pero sin aumento significativo adicional entre las 2 y 3 semanas de hipoxia. Tres semanas de normoxia luego de 3 semanas de hipoxia revirtieron la hipervascularización inducida por hipoxia. Esta hipervascularización se asoció con una densidad incrementada de la proteína transportadora de glucosa (GLUT-1) en microvasos cerebrales aislados. esta expresión incrementada de GLUT-1 fue evidente luego de 1 semana de hipoxia, permaneció constante luego de 3 semanas y no revirtió luego de 3 semanas de recuperación normóxica. Así, a diferencia de la vascularización cerebral incrementada inducida por hipoxia, la densidad de GLUT-1 incrementada inducida por hipoxia en microvasos cerebrales aislados no es rápidamente reversible. La combinación de vascularización cerebral incrementada y la expresión incrementada de GLUT-1 en los microvasos cerebrales de ratas sometidas a hipoxia hipobárica se asocian con un transporte incrementado de glucosa de la sangre al cerebro y una tasa metabólica cerebral regional de glucosa incrementada. Estos hallazgos documentan varias respuestas adaptativas a la hipoxia prolongada y sugieren una glicólisis incrementada en el cerebro de ratas adultas luego de hipoxia moderada prolongada.

Palabras claves: Hipoxia, Microcirculación, Cerebro, Glicólisis.

RÉSUMÉ: Adaptation de la microcirculation cérébrale à l'hypoxie hypobare prolongée.

Quelques effets de l'hypoxie hypobare modérée (0,5 atm) sur la microcirculation cérébrale de rats adultes ont été revus. La vascularisation cérébrale augmente à la suite d'une hypoxie prolongée, mais le déroulement dans le temps de la réponse adaptative et sa réversibilité ne sont pas connus. Au bout de 4 jours d'hypoxie aucune augmentation significative de la vascularisation cérébrale n'a été observée, mais au bout d'une semaine on a pu noter une augmentation d'environ 33 %. Cette vascularisation a continué à se développer jusqu'à un maximum d'environ 70 % par rapport aux valeurs de référence, après 2 semaines d'hypoxie, mais sans accroissement supplémentaire significatif entre la 2e et la 3e semaine. Au bout de 3 semaines de normoxie faisant suite à 3 semaines d'hypoxie, la vascularisation cérébrale est redevenue normale. Cette hypervascularisation induite par hypoxie a été associée à une densité accrue de la protéïne transportant le glucose (GLUT-1) dans des vaisseaux capillaires cérébraux isolés. Cet accroissement de densité de GLUT-1 dans ces capillaires a été notoire après 1 semaine d'hypoxie, se maintenant à ce niveau après 3 semaines d'hypoxie et 3 semaines de récupération normoxique. Ainsi, à la différence de la vascularisation cérébrale induite par hypoxie, l'accroissement de la densité de GLUT-1 induit par hypoxie dans les vaisseaux capillaires cérébraux isolés n'est pas rapidement réversible. La combinaison de la vascularisation cérébrale et de l'expression accrue de GLUT-1 dans les vaisseaux cérébraux de rats soumis à l'hypoxie hypobare est associée à un transport accru du glucose du sang au cerveau, accompagné d'une élévation des concentrations cérébrales de glucose et de lactate et d'un taux métabolique cérébral régional accru du glucose. Ces découvertes expliquent plusieurs réponses adaptatives à l'hypoxie prolongée et suggèrent une glycolyse accrue dans le cerveau de rats adultes après une hypoxie modérée et prolongée.

Mots-clés : Hypoxie, Adaptation, Microcirculation, Cerveau, Glycolyse.

SUMMARY: We review some of the effects of moderate hypobaric hypoxia (0.5 atmosphere} on the brain microcirculation of adult rats. Brain vascuiarity increases after prolonged hypoxia, but the time course of the adaptive response and its reversibility. were not known. We found no significant increase in brain vascularity after 4 days of hypoxia but noted a significant increase of about 33% after 1 week. Cerebral vascutarity continued to increase, attaining a maximum of about 70%, over control values afler 2 weeks of hypoxia, but without significant further increase between 2 and 3 weeks of hypoxia. Three weeks of normoxia afler 3 weeks of hypoxia reversed the hypoxia-induced cerebral hypervascularity. The cerebral hypervascularity induced by hypoxia was associated with an increased density of the glucose transported protein (GLUT-1) in isolated cerebral microvessels. This increased expression of GLUT-1 in cerebral microvessels was evident at 1 week of hypoxia, remained constant after 3 weeks of hypoxia, and was not reversed after 3 weeks of normoxic recovery. Thus, unlike the hypoxia-induced increased brain vascularity, the hypoxia-induced increased density of GLUT-1 in isolated cerebral microvessels is not readily reversible. The combination of increased brain vascularity and increased GLUT-1 expression in cerebral microlvessels of rats subjected to hypobaric hypoxia is associated with increased blood-to-brain glucose transport, increased brain concentrations of glucose and lactate, and increased regional cerebral metabolic rate for glucose. These findings document several adaptive responses to prolonged hypoxia and suggest increased glycolysis in the adult rat brain after moderate and prolonged hypoxia.

Key Words.: Hypoxia, Adaptation, Microcirculation, Brain, Glicolysis

INTRODUCTION

The mammalian brain requires uninterrupted perfusion for its normal functions. Blood flow to the brain delivers primarily oxygen and glucose; and every clinician knows the dire consequences of severe brain hypoxia. When hypoxia is brief and moderate, regional brain oxygen tension may be maintained by increasing cerebral blood flow, which is associated with shorter capillary mean transit time, increased red cell velocity, and possible capillary recruitment. However, in prolonged moderate hypoxia, other adaptive (or maladaptive) mechanisms become manifest (1-3). These differ by species, but the rat's response is similar to man's and for that reason, it is believed that studies of chronic moderate hypoxia in the rat are of clinical relevance (2). Chief among the adaptive mechanisms to prolonged moderate hypoxia are increased red cell mass and changes in ventilation and arterial blood gases, both of which increase the oxygen carrying capacity of the blood. In fact, LaManna *et* al. (4) examined rats exposed to hypobaric hypoxia at 0.5 átmosphere (ATM) for 3 weeks and found that although cerebral blood flow in hypoxic rats was not significantly increased, the marked increase in arterial hematocrit allowed similar oxygen delivery to the brains of hypoxic rats as that to the brains of normoxic controls. However, if the increased hematocrit and hyperventilation were the only adaptive responses to hypoxia, brain oxygen tension in hypoxic rats would have remained low because of the lower capillary PO2 which is the driving force for oxygen diffusion into tissues. Thus, increased hematocrit and hyperventilation are not sufficient for adequate brain oxygenation; other adaptive responses are needed.

Over the last few decades, a considerable body of experimental evidence led to the observations that the brain's vascularity increases in prolonged hypoxia, presumably yielding smaller intercapillary distances which would improve oxygenation (4-9). The main aim here is to summarize pertinent results from our laboratory concerning the alterations in the cerebral microcirculation that take place in adult rats subjected to hypobaric hypoxia at 0.5 ATM for periods of up to 3 weeks. Because of the substantial enrichment of brain capillaries with the glucose transporter protein, GLUT-1 (10), we will also review the effects of hypoxia on GLUT-1 in cerebral microvessels, and therefore on blood-to-brain glucose transport and brain glucose metabolism.

In all experiments, adult male Wistar rats aged 3-6 months were kept in hypobaric chambers maintained at 0.5 ATM (380 torr) for the indicated times except for 1 hour per day when the pressure was returned over 10 minutes to atmospheric for cage cleaning and for water and food replenishment (4). In some experiments, the reversibility of these changes were studied in rats that were subjected to hypoxia for 3 weeks but then were allowed to recover at normal atmospheric pressure for another 3 weeks. In all experiments, hypoxic rats were compared to normoxic littermates that were kept outside the hypobaric chamber, but which were treated in an otherwise identical manner.

CHANGES IN CEREBRAL VASCULARITY

LaManna *et* al. previously reported increased brain vascularity after 3 weeks of hypoxia in the same experimental hypoxia model that was described above (4). The increased vascularity ranged from 40 to 70% above control regional values that were measured in coronal brain sections that were stained for alkaline phosphatase as a marker of cerebral capillaries. However, the time course of the development of increased brain vascularity and the reversibility of this response remained unknown.

Harik *et al.* measured cerebral vascularity in rats that were subjected to hypoxia for periods of 1, 2, or 3 weeks and in rats subjected to 3 weeks of hypoxia but then allowed to recover for another 3 weeks (11). For measurement of cerebral vascularity, the rats were anesthetized, perfused-fixed *in situ*, and coronal brain sections were immunostained using GLUT-1 antibody and the peroxidase-antiperoxidase method as described previously (12). Two regions representing the frontal motor cortex were studied where microvessel profiles were counted and exoressed as microvessel profiles/ mm^2 of cerebral cortex as described before (4).

Hypoxic rats developed polycythemia within days of hypoxic exposure (Fig. 1). Significant differences in the hematocrit between hypoxic and normoxic rats was evident at 1, 2, and 3 weeks of hypoxia (11). Three weeks after normoxic recovery, the hematocrit returned to control values indicating full reversibility of the hypoxia-induced polycythemia (Fig. 1).

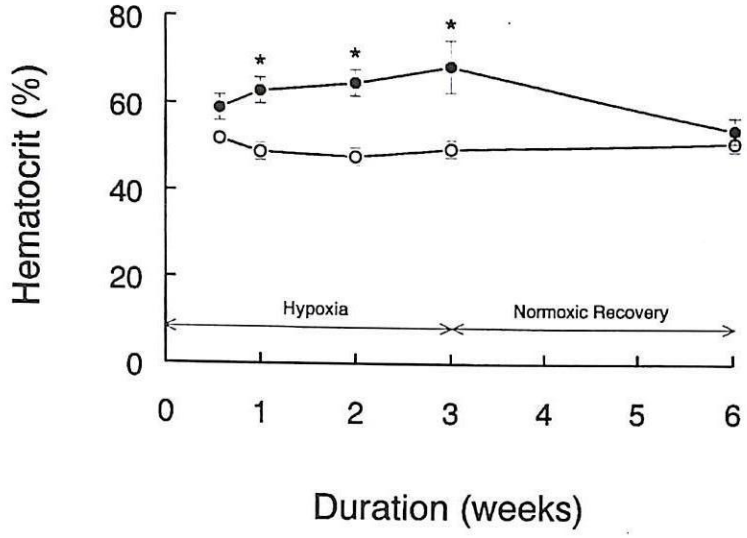

Figure 1. Effects of hypoxia and recovery from hypoxia on hematocrit.
The venous hematocrit in normoxic (o) and hypoxic (·) rats is plotted as a function of experimental duration. The data represent means ±SD of 3 rats in each group at 4 days, 20 rats in each group at 1 week, 3 rats in each group at 2 weeks, 20 rats in each group at 3 weeks and 6 rats in each group at 6 weeks. The hematocrit was significantly increased in the hypoxic group at 1 week, 2 weeks and 3 weeks, but there was no significant difference in the hematocrit values at 6 weeks. Taken from ref. 11.

On the other hand, cerebral vascularity was not altered significantly after 4 days of hypoxia (Fig. 2). After one week of hypoxia, there was a 33 % increase in cerebral vascularity which continued to increase attaining a maximum of about 70% above control values after 2 weeks of hypoxia (Fig. 2). The increased cerebral microvessel density like polycythemia, was reversible in rats that were allowed to recover for 3 weeks (11).

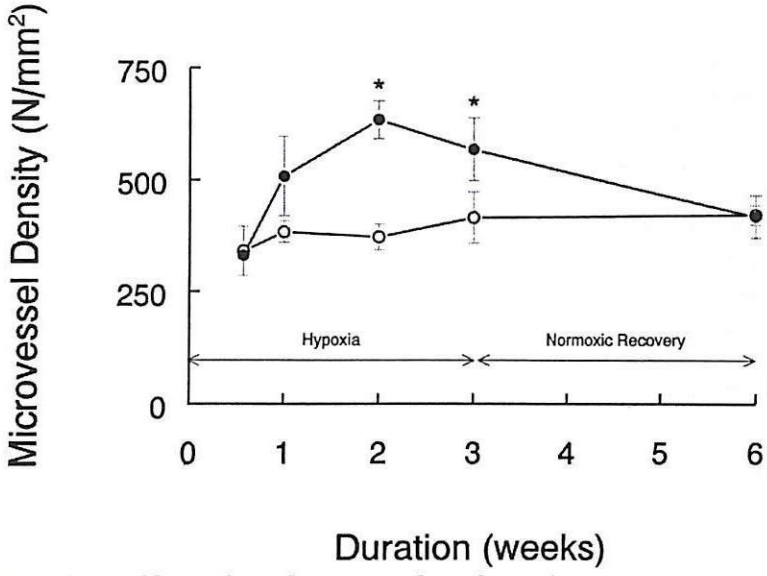

Figure 2. Effects of hypoxia and recovery from hypoxia on cerebral vascularity.
The cerebral microvessel density in normoxic (o) and hypoxic (·) rats as a function of the experimental duration. The data represent ±SD of 3 rats in each group at each time point. Analysis of variance showed an effect of hypoxia on the cortical microvessel density at 2 weeks and 3 weeks of hypoxia but not at the other experimental durations. There was no effect of duration on the cortical microvessel density in control rats. There was an effect of time on cerebral microvessel density in hypoxic rats in that the cerebral vascular density increased significantly between 4 days and 7 days of hypoxia ($P<0.001$) and decreased significantly during the 3 week recovery period ($P<0.04$). Taken from ref. 11.

These results from two dimensional studies of the effects of moderate prolonged hypoxia on brain vascularity are compatible with the results of investigations using three dimensional geometry of the microvascular network in the cerebral cortex of rats exposed to the same hypoxic insult as reported by Mironov et al. (13). In that study, the vascular pattern in hypoxic rats was similar to that of normoxic rats except that there was increased vascular density, tortuosity and loop-like formation, which were most evident in the deeper part of the cerebral cortex. Quantitative assessment of the mean capillary segment length showed a significant increase in the deep parietal cortex of hypoxic rats. The increased capillary segment length in the deep parietal cortex of hypoxic rats was also evident in comparisons of the frequency distribution of capillary segment lengths (13). In hypoxia, the peak frequency was significantly increased from 37.1 ± 1.7 pm to 52.3 ± 1.9 pm (means ± SD). If sprouting was the only mechanism of the increased vascularity of the hypoxic cerebral cortex, then a smaller capillary segment length would be predicted. The findings to the contrary indicate elements of capillary elongation and hypertrophy which are induced by hypoxia.

CHANGES IN GLUCOSE TRANSPORT AT THE BLOOD-BRAIN BARRIER

Brain capillaries are known to have a high density of the glucose transporter protein (GLUT-1) in their plasma membranes (9-12). This is understandable since brain capillaries, which constitute less than 0.5 % of the wet weight of the brain, have to transport glucose for the overwhelming mass of surrounding neurons and glia. Because oxygen, but not glucose, is limited in hypobaric hypoxia, one would suspect that newly formed brain capillaries would have a lower GLUT-1 density in their plasma membranes to avoid a mismatch between oxygen and glucose delivery.

Harik et al. quantitated GLUT-1 in isolated cerebral microvessels obtained from rats subjected to hypobaric hypoxia for 1 or 3 weeks, and in their littermate controls (9). The isolated cerebral microvessels were assayed for their D-glucose-displaceable cytochalasin-B binding. The results showed that the maximal number of cytochalasin-B binding sites was significantly increased by about 25 % at 1 and at 3 weeks of hypoxia while cytochalasin-B binding to particulate fractions of the cerebral cortex was not affected by hypoxia.

Because cytochalasin-B binds to numerous glucose transporters, and is not specific to GLUT-1, and because the normoxic reversibility of this phenomenon was not studied, Harik et al. recently measured GLUT-1 in isolated cerebral microvessels from rats subjected to various periods of hypoxia with and without recovery, using quantitative immunological determination of GLUT-1 by Western blots (11). The effect of hypoxia on the protein expression of GLUT1 in isolated cerebral microvessels was calculated as the ratio of the optical density of the autoradiograms of the GLUT-1 band in Western blots of hypoxic and normoxic samples of microvessels from littermate rat groups that were assayed simultaneously (11). The expression of GLUT-1 in isolated cerebral microvessels was significantly increased after 1 and 3 weeks of hypobaric hypoxia (Fig. 3). However, the increased expression of GLUT-1 in cerebral microvessels isolated from hypoxic rats remained significantly higher than control levels after 3 weeks of normoxic recovery (Fig. 3). Thus, it appears that the increased density of GLUT-1 in isolated cerebral microvessels is not easily reversible (11).

The results obtained by the Western blot immunochemical method for quantitating the density of GLUT-1 are consistent with those measured in the earlier study (9) where cytochalasin-B binding was used to quantitate the glucose transporter in isolated cerebral microvessels. Both studies showed about 50% increase in the density of the glucose transporter in the cerebral microvessels of rats subjected to hypoxia. This was not unexpected given the fact that GLUT-1 is the major, if not the only, glucose transporter in cerebral microvessels. These results are consistent with the increased blood-to-brain glucose transport in vivo in rats that were exposed to hypobaric hypoxia (9). In contradistinction to the decline in the cerebral vascular density in hypoxic rats that were allowed 3 weeks of normoxic recovery, the GLUT-1 density in isolated cerebral microvessels of recovering rats did not decline (11). This suggests that either the half life of GLUT-1 in cerebral microvessels is prolonged so that GLUT-1 persists for extended time periods, or that the stimulus underlying GLUT-1 synthesis does not shut off easily.

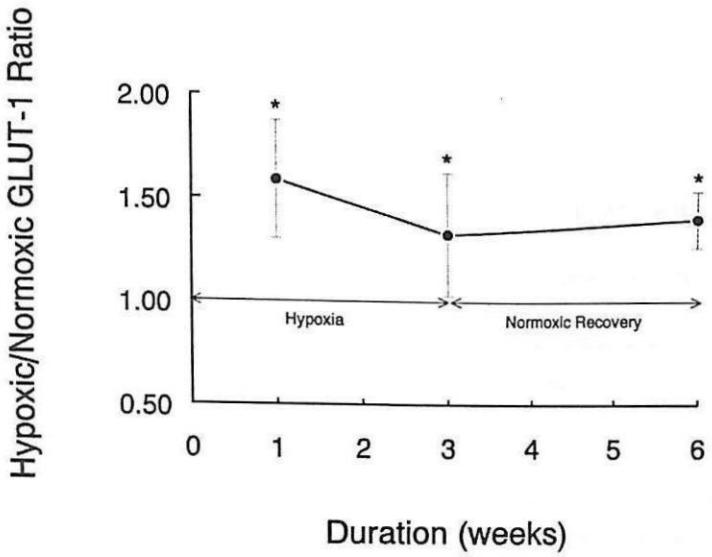

Figure 3. Effect of hypoxia on GLUT-1 density in isolated cerebral microvessels.
GLUT-1 density ratio of hypoxic:normoxic preparations as a function of the experimental duration. The data represent means ± SD of 3 microvessel preparations after 1 week, 5 preparations after 3 weeks, and 4 preparations after 6 weeks. The Student t-test showed a significant effect of hypoxia at all 3 time periods that were studied ($P < 0.05$), but no effect of duration of hypoxia or recovery by analysis of variance. Taken from ref. 11.

The combination of the increased brain vascularity and the increased density of GLUT-1 in cerebral microvessels of rats subjected to hypobaric hypoxia was found to be associated with increased blood-to-brain glucose transport in vivo in rats subjected to 3 weeks of the same hypobaric condition described above (9). Regional blood-to-brain D-glucose transport and blood flow were determined by the double-label, single-pass indicator-fractionation atrial bolus injection method. Cerebral blood flow was slightly higher in hypoxic rats but the difference did not reach significance in any of the brain regions. Also, there were no significant alterations in regional brain L-glucose space in hypoxic rats. The extraction fraction values of D-glucose were increased by about three-fold in hypoxic rats and the unidirectional blood-to brain influx was doubled in those rats. That the increased brain glucose influx in hypoxia was not commensurate with the increased extraction fraction despite the similar regional blood flow and the similar L-glucose space in both groups of rats is probably a reflection of the markedly decreased blood plasma fraction which is a consequence of the increased hematocrit and the decreased blood plasma fraction in hypoxic rats. The calculated maximal transport capacity was significantly increased in hypoxic rats (9).

Thus, these findings indicate that in addition to the increased vascularity of the brain in hypobaric hypoxia, there is an increased density of the glucose transporter moiety at the blood brain barrier which contributes significantly to the increased unidirectional transport of glucose into the brain. The physiological concomitants of these observations have been followed up in other studies that were performed using the same model of hypobaric hypoxia. Harik et al. measured regional brain concentrations of glucose, glycogen, lactate, adenosine triphosphate, phosphocreatine, regional intracellular pH and regional cerebral metabolic rate for glucose in rats subjected to 3 weeks of hypobaric hypoxia (14). The results obtained from that work indicated a significant increase in brain glucose and lactate levels and a significant decrease in brain glycogen levels in hypoxic rats but without a significant change in brain ATP, phosphocreatine, and pH values. Thus, the increased density of the glucose transporter at the blood-brain barrier, and the increased blood-to-brain glucose transport result in higher brain glucose concentrations. The lack of differences between hypoxic and normoxic brains in their high energy phosphate content further indicates that at this level of hypoxia, there is adequate compensation for oxygen lack. The similar regional brain intracellular pH in hypoxia and normoxia is another indication of the compensation which prevents tissue acidosis in the face of higher lactate concentrations in the brains of hypoxic rats. Harik et al. speculated that the increased brain lactate levels in hypoxia are useful for maintaining brain pH in the presence

of low brain tissue CO_2 tension, thereby avoiding the necessity to export brain bicarbonate (14).

The concomitant findings of decreased brain glycogen and increased brain lactate suggest increased anaerobic glycolysis in the hypobaric brain. The other important finding was the increased cerebral metabolic rate for glucose in hypoxic rats (14). The authors suspected that the increased cerebral metabolic rate for glucose in hypoxia, presumably for anaerobic glycolysis, combined with the low brain plasma flow in hypoxic rats and the fact that rats erythrocytes do not carry glucose, are logical explanations for the increased glucose transport at the bloodbrain barrier. The exact mechanism of how the higher cerebral metabolic rate for glucose in prolonged moderate hypoxia eventually causes up-regulation of glucose transport at the blood-brain barrier remains unknown.

The increased reliance of the brain on possible anaerobic glucose metabolism is probably one of several compensatory mechanisms that underlie adaptation to prolonged hypoxia which was previously suspected in the heart (15). Perhaps this is the reason why a carbohydrate rich diet is believed to be beneficial for subjects exposed to high altitude (16).

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