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EFFECTS OF ISCHEMIA/ REPERFUSION ON THE PHOSPHOTYROSINE PROTEINS CONTENTS IN HIPPOCAMPUS

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ABSTRACT

Aim and Methods: The effects of three drugs including ketamine (KT), a noncompetitive antagonist of NMDA receptor (NR), nifedipine (ND), a voltage gated calcium channel (VGCC) antagonist and 6, 7-dinitroquinoxaline-2, 3-dione (DNQX), a non-NMDA receptor antagonist on the contents of phospho-tyrosine proteins (p-tyr-pr) in the synaptosomal (P₂), the crude membrane (P₃), and the cytosolic (S₃) fractions of hippocampus in forebrain ischemia of mongolian gerbils were studied. **Results:** the contents of p-tyr-pr in all three fractions (P₂, P₃, S₃) decreased 15 min after ischemia, but the contents of p-tyr-pr in S₃ fraction decreased more obviously than the others did. With the increase of reperfusion time, the contents of p-tyr-pr in all of these fractions recovered gradually, but the p-tyr-pr in S₃ fraction increased more rapidly among them, in the P₂ fraction, the contents of p-tyr-pr increased slowly, but significantly and sustained longer during reperfusion when compared with that of P₃ did. The increase in p-tyr-pr contents induced by cerebral ischemia/ reperfusion was partially antagonized by KT and ND administration prior to cerebral ischemia, under these conditions, DNQX has no effect on it. **Conclusion:** the increase of p-tyr-pr contents induced by cerebral ischemia/ reperfusion is related to NR channel and L-type VGCC, but not to non-NR channel.

KEY WORDS: brain ischemia; p-tyr-pr; KT; ND; NR; L-type VGCC

模拟急性低氧大鼠血清 T₃, T₄ 和下丘脑 TRH 含量变化

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高原急性影响中, 出现体重暂时性下降, 食欲减退等现象。随习服的产生, 代谢变化明显减弱或消失。人类在 5 000 m 以上时代谢变化变得更为明显并不断发展则体脂和蛋白储备明显减少, 血液中红细胞大量增加, 血液粘稠度增大等。与此同时也产生一系列神经内分泌改变。下丘脑-腺垂体-甲状腺轴是调节体内代谢的重要因素之一, 因而 HPT 轴低氧应答的研究对低氧习服和内环境的适应性反应有着重要价值。

1 材料和方法

健康雄性 Wistar 大鼠体重 (140 ± 10) g, 鼠源系中国科学院上海实验动物中心, 在中科院西北高原生物研究所动物饲养室繁殖多代。实验温度 18℃。低氧模拟: 使用减压舱模拟高原高海拔低氧的方法, 以实验地西宁海拔 2 300 m 为对照, 观察两种高海拔即 5 000 m (54.02 kPa), 7 000 m (41.04 kPa) 对大鼠的影响。实验动物随机分组, 放入减压舱, 以 140 m/min 速度升至所需海拔高度: 动物于舱内可自由取食和饮水。各低氧组大鼠在减压舱中分别暴露在两种模拟海拔的持续时间分别为 1 h, 3 h, 24 h。上午 10 时左右迅速断头取血, 分离下丘脑放入液氮。血清 T₃, T₄ RIA 测定使用中国原子能科学院北京同位素研究所放免药盒。组织 TRH RIA 测定使用北京北方生物技术研究所放免药盒。组织 TRH 提取: 参照 Brownstein 等方法, 0.01 mol/L 磷酸缓冲液和 0.15 mol/L NaCl 中匀浆, 95% 甲醇提取, 10 000 r/min 低温离心 30 min。上清液加温去甲醇, 提取物放入 0.25% 牛血清白蛋白, 0.01 mol/L 磷酸缓冲液和 0.15 mol/L NaCl 中 -40℃ 保存。下丘脑蛋白定量使用 Lowry 法。质控所用 TRH 购自 Sigma 公司, 其它试剂均采用国内产品。所有实验数据以均数 ± 标准误 (均 ± 标准误) 表示, 采用 *t* 检验 (*t*-test) 检验差异显著性。

2 结果

血清 T₃, T₄ 变化如表 1。所有低氧组均低于对照。5 000 m 1 h, 3 h, 24 h 低氧组血清 T₃ 含量分别为对照的 81.4%、51.5%、46.1%。7 000 m 1 h, 3 h, 24 h 组分别为对照的 82.8%、49.8%、32.1%。5 000 m 1 h, 3 h, 24 h 组血清 T₄ 含量分别为对照的 80.9%、76.7%、54.8%。7 000 m 1 h, 3 h, 24 h 组分别为对照的 65.6%、72.8%、44.3%。 (下转第 71 页)

The murine stromal cell line AFT024 acts specifically on human CD34 + CD38 - progenitors to maintain primitive

function and immunophenotype *in vitro* [J]. *Exp Hematol*, 1998, 26:612-619.

THE TRANSFECTION AND EXPRESSION OF RETROVIRAL-MEDIATED HUMAN FL cDNA IN BONE MARROW STROMAL CELL LINE

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ABSTRACT

Aim: The expression of retroviral-mediated FL gene transfer into bone marrow stromal cell line HFCL was studied. **Methods:** FL T3 ligand (FL) cDNA was recombined with retroviral vector pLXSN by gene recombination technology. The recombinant plasmid was transferred into retrovirus packaging cell line PA3 17 by lipofectamine, and the resistant clones were selected by G418 selective medium. The mRNA expression in HFCL cells and integration of genome DNA were assayed by RT-PCR and genomic DNA PCR. The biological activity of FL in the culture was investigated by mouse bone marrow CFU-GM assay. **Results:** The recombinant plasmid pLFSN was successfully constructed. The expression of FL mRNA was detected in HFCL cells. In the genome of these infected target cells, neo gene and FL cDNA were successfully expressed. The biological activity of FL in the culture demonstrated that HFCL cells transfected with FL could significantly augment FL *in vitro*. **Conclusion:** These results suggest that bone marrow stromal cell lines might become target cells of gene therapy.

KEY WORDS: retroviral vector; bone marrow stromal cell line; FL T3 ligand; gene expression

(上接第 9 页)

Tab. 1 Effects of acute hypoxia on serum T₃、T₄ of rats (均 ± 标准差, n = 5 or 6)

	Control	5 000 m			7 000 m		
		1 h	3 h	24 h	1 h	3 h	24 h
Serum T ₃ (mg/L)	0.978 ± 0.060	0.796 ± 0.049 *	0.504 ± 0.029 **	0.451 ± 0.042 **	0.810 ± 0.028 **	0.487 ± 0.039 **	0.314 ± 0.017 **
Serum T ₄ (mg/L)	69.433 ± 4.622	56.171 ± 2.094 *	53.258 ± 3.513 *	38.054 ± 3.852 **	45.520 ± 3.934 **	50.558 ± 5.201 *	30.767 ± 4.403 **

*P < 0.05, **P < 0.01, vs control

急性模拟低氧 5 000 m 24 h 组和 7 000 m 1 h、3 h 组下丘脑 TRH 含量明显高于对照组(0.277 ± 0.023) ng/mg Pro 分别为(0.387 ± 0.022) ng/mg Pro (P < 0.01)、(0.424 ± 0.015) ng/mg Pro (P < 0.01)、(0.381 ± 0.024) ng/mg Pro (P < 0.01)。5 000 m 1 h、3 h 与 7 000 m 24 h 组与对照均无显著差异,分别为(0.314 ± 0.042) ng/mg Pro (P > 0.01)、(0.306 ± 0.018) ng/mg Pro (P > 0.01)、(0.328 ± 0.016) ng/mg Pro (P > 0.01)。

3 讨论

许多研究表明,一般除低温应激引起大鼠下丘脑-垂体-甲状腺轴的活动加强以外,大多应激对其产生抑制性效应。本实验观察结果,模拟海拔 5 000 m 和 7 000 m 低氧 1 h、3 h、24 h,均引起循环 T₃、T₄ 水平暂时性低下。且随低氧的强度增加和暴露时间的延长,循环 T₃、T₄ 水平更进一步降低。高海拔低氧环境对机体代谢产生影响,有资料显示,长期居住在高原高海拔环境的人类或动物氧的总耗量降低,这可能是适应性反应的一种形式。甲状腺素在调节基础代谢、蛋白质和脂肪代谢中起重要作用,高原低氧代谢变化的调节可能有甲状腺素参与。在海拔 4 200 m 地区久居的外来人员基础代谢率低于海平面的 4 ~ 5%。高海拔地区移居和世居人体血清胆固醇增高,大鼠在模拟急性低氧暴露 24 h 后,血浆甘油三酯和胆固醇含量也高于对照组。这都提示与甲状腺功能减退相吻合。排除高原低氧环境中温度对机体的影响外,循环中 T₃、T₄ 含量的降低可能使机体在静息状态下维持较低的代谢率而免于代谢过度消耗。

在 5 000 m 和 7 000 m 不同低氧条件下,下丘脑 TRH 的变化可能与下丘脑中 TRH 的分泌与合成有关有报导观察到,模拟 5 000 m 慢性低氧引起大鼠下丘脑室旁核 TRH mRNA 合成抑制,但急性低氧暴露 24 h 小时,室旁核 TRH mRNA 合成未见改变。因此 5 000 m 24 h 下丘脑 TRH 含量升高可能是 TRH 的分泌减少所致。7 000 m 1 h、3 h 低氧暴露,下丘脑 TRH 的分泌即产生较强的抑制反应。7 000 m 24 h 低氧过程中,随时间延长下丘脑 TRH 分泌进一步抑制而合成抑制逐渐开始,致使 7 000 m 24 h 组下丘脑 TRH 含量接近对照水平。因此,在大鼠低氧暴露过程中,血清 T₃、T₄ 含量的暂时性低下可能与中核 TRH 分泌抑制有着一定的相关性。

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