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厦门大学

硕 士 学 位 论 文

间歇性低氧对新生小鼠脑神经发生和 pax6 表达的影响

The effects of intermittent hypoxia on neurogenesis and *pax6* expression in the neonatal mice

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缩略词索引

英文缩写	英文全名	中文全名
3V	3rd ventricle	第三脑室
aca	anterior commissure, anterior part	前联合前部
Aco	anterior cortical amygdaloid nucleus	前皮质杏仁核
AOM	anterior olfactory nucleus, medial part	前嗅觉核中间部分
AOP	anterior olfactory nucleus, posterior part	前嗅觉核后部分
alv	alveus of the hippocampus	海马槽
Astr	amygdalostriatal transition area	杏仁-纹状体过渡区
BLA	basolateral amygdaloid nucleus, anterior part	外侧杏仁核前部
BLV	basolateral amygdaloid nucleus, ventral part	外侧杏仁核腹侧
BMA	basomedial amygdaloid nucleus, anterior part	内侧杏仁核前部
BMP	basomedial amygdaloid nucleus, posterior part	内侧杏仁核后部
BPC	basal progenitor cell	基底祖细胞
BSTAI	bed nucleus of the stria terminalis，intraamygdaloid division	床核终纹内侧杏仁核分区
BSTLD	bed nucleus of the stria terminalis,lateral division, dorsal part	床核终纹，侧腹部
CP	cortical plate	大脑皮质
DG	dentate gyrus	齿状回
Di	diencephalons	间脑
E	embryonic day	胚胎天数
Ec	external capsule	外囊
FB	forebrain	前脑
FC	fasciola cinereum	灰质细带
HB	hindbrain	后脑
HD	homeodomain	同源区

英文缩写	英文全名	中文全名
IM	intermediate zone	中间区
IPC	intermediate progenitor cell	中间先祖细胞
LV	lateral ventricle	侧脑室
LSI	lateral septal nucleus, intermediate part	横向鼻中隔中部分
LSV	lateral septal nucleus, ventral part	横向鼻中隔和腹侧
LPO	lateral preoptic area	视叶外侧
LH	lateral hypothalamic area	下丘脑外侧
LDVL	laterodorsal thalamic nucleus,ventrolateral part	腹外侧丘脑
LMol	lacunosum moleculare layer of the hippocampus	海马分子层
M	motor cortex	运动皮层
MB	midbrain	中脑
MePD	medial amygdaloid nucleus, posterodorsal part	内侧杏仁核后背部
MePV	medial amygdaloid nucleus	内侧杏仁核
MM	medial mammillary nucleus, medial part	乳头体内侧核
MPOM	medial preoptic nucleus, medial part	视叶内侧核，内部
MGP	medial globus pallidus	内侧苍白球
Mtu	medial tuberal nucleus	内侧结节核
NSCs	nureal stem cells	神经干细胞
Ns	nigrostriatal bundle	黑质纹状体
OB	olfactory bulb	嗅球
Or	oriens layer of the hippocampus	海马始层
OV	olfactory ventricle	嗅室
PD	paired domain	配对结构域
pir	piriform cortex	梨状皮层
Pe	periventricular hypothalamic nucleus	脑室周下丘脑核
PLCo	posterolateral cortical amygdaloid nucleus	后外侧皮质杏仁核
PMCo	posteromedial cortical amygdaloid nucleus	后中皮质杏仁核
PH	posterior hypothalamic area	后下丘脑

英文缩写	英文全名	中文全名
PS	pia surface	脑膜表面
PST	Pro/Ser/Thr-rich region	富含 Pro-Ser-Thr 区
r	Rhombomere	菱脑原节
RGC	radial glial cell	放射状质细胞
RPF	retroparafascicular nucleus	顶盖前区后联合
st	stria terminalis	终纹
SubG	subgeniculate nucleus	拟膝核
SVZ	subventricular zone	脑管膜下区
tel	telencephalon	端脑
Tu	olfactory tubercle	嗅结节
VDB	nucleus of the vertical limb of the diagonal band	斜角带垂直枝核
VP	ventral pallidum	腹侧苍白球、黑质
VPM	ventral posteromedial thalamic nucleus	腹侧后中下丘脑核
VM	ventromedial thalamic nucleus	丘脑腹内侧核
VPL	ventromedial thalamic nucleus	腹后外侧丘脑核
VMH	ventromedial hypothalamic nucleus	下丘脑腹内侧核
VMHDM	ventromedial hypothalamic nucleus ,dorsomedial part	腹内侧下丘脑核背部
VS	ventricular surface	脑室表面
VZ	ventricular zone	脑室区
ZIV	zona incerta, ventral part	未定义区腹侧
ZID	zona incerta, dorsal part	未定义区背侧

摘 要

目的

我们前期研究发现，出生后间歇性低压低氧暴露能够增强小鼠空间记忆，但同时损害联合记忆。已知低氧能够诱导神经发生，而转录因子 Pax6（paired homebox 6）在胚胎神经系统发育过程中能够调节神经细胞的增殖和分化。因此本研究观察出间歇性低氧对新生小鼠脑神经发生和 Pax6 表达的影响，以期探明其对记忆影响的神经机制。

材料和方法

新生小鼠出生后立即分别暴露于模拟高原海拔 2 千米(16.0%O_2；PaO_2, 60mmHg) 和 5 千米 (10.8% O_2；PaO_2, 41mmHg)低压低氧环境，每天低氧暴露 4 小时，连续低氧 4 周。低氧结束后小鼠常氧环境分组饲养 1 周和 4 周。对照组为不低氧暴露小鼠（设为海平面，21% O_2）。为了观察神经发生情况，低氧结束后连续 3 天注射 5-溴脱氧尿嘧啶核苷（5-Bromo-2-deoxyUridine，BrdU），每天一次；为了观察新生神经细胞是否能够与原有神经细胞建立联系，每组小鼠在处死前 2 小时给予足底电刺激，免疫荧光方法用来观察 BrdU 和 c-fos 在脑内的表达情况并进行定位分析；为了观察低氧对 Pax6 表达的影响，分别用免疫荧光和 western-blot 技术对 Pax6 在脑内表达情况进行定位和定量分析。

结果

1. 低氧对神经发生的影响

（1）与对照组相比，低氧暴露 4 周小鼠在脑内 OV、LV、pir 、Or、DG、门、丘脑和运动皮质等区域存在 BrdU 阳性细胞，而且 2 千米低氧处理组的 BrdU 阳性细胞明显比 5 千米低氧处理组的 BrdU 阳性细胞多。

（2）进一步研究发现，BrdU 与 NeuN 和 GFAP 都共存。

（3）BrdU 阳性细胞可以持续存活到低氧结束后 4 周。

（4）低氧结束后 4 周，发现 c-fos 表达于新生的 NeuN 阳性细胞。

2. 低氧对 Pax6 表达的影响

（1）在正常对照 30 天龄小鼠中，Pax6 在多个脑区表达，包括 OV、VBD、VP、LV、pir、M、aca、Tu、LSI、LSV、BSTLD、MPOM、LPO、3V、Pe、LH、VMH、BLA、BMA、Aco、BLV、st、LDVL、MGP、VMHDM、alv、Or、PLCo、PMCo、Mtu、BSTAI、MePD、MePV、PH、ns、ec、AStr、BMP、VPM、VM、VPL、DG、LMol、FC、SubG、RPF、ZIV、ZID、MM。

（2）低氧 4 周结束后（出生后 30 天），模拟高原 2 千米低压低氧处理组表达 Pax6 的核团有 OV、Pir、LV、DG、MTu、BMP 和 PLCo；模拟高原 5 千米低压低氧处理的实验动物中表达 Pax6 的区域有 OV、pir、Or、Tu、M、VDB、LV、VP、LSI、LSV、MPOM、DM、MCPO、PLCo、MGP、Pe、LH、VMH、BMA、BLA、LDVL 和 MePD。

（3）低氧暴露 4 周后，Pax6 与 NeuN 和 GFAP 都共存表达，并且小鼠脑内可见 Pax6 与 BrdU 共染。

（4）Western-blot 定量结果显示：① 横向比较发现，在低氧结束后和低氧结束后 1 周，与对照组比较，2 千米和 5 千米低氧暴露小鼠（244,544 和 245,545 表示）的 Pax6 表达量显著减少；② 纵向比较低氧结束后、低氧结束后 1 周和低氧结束后 4 周小鼠发现，对照组、2 千米低氧组和 5 千米低氧组脑内 Pax6 的表达量都逐渐增加。相对于对照组的增加，5 千米低氧出生后 8 周处死实验动物（548 表示）Pax6 表达量比对照组 8 周处死实验动物（048 表示）显著增多。

结论

1.出生后间歇性低压低氧暴露可以促进小鼠脑神经发生，这种新发生的细胞可以同时分化为神经细胞和星形胶质细胞。新发生的细胞可以长时间存活，并可与已有的成熟神经细胞建立神经环路连接。

2．出生后脑内仍然有大量脑区的神经细胞和胶质细胞都表达 Pax6。出生后

低氧显著减少 Pax6 在脑内多个区域的表达。

3. 在低氧暴露小鼠脑内，Pax6 可见与 BrdU 共同表达于 NeuN 阳性细胞，提示 Pax6 可能参与了低氧诱导神经细胞的发生。

关键词： 间歇性低氧； 神经发生；Pax6

Abstract

Objectives

Our previous study found that intermittent hypoxia enhanced spatial memory while depressed the associative memory. It is well known that hypoxia could induce neurogenesis, and the Pax6(paired homebox 6）plays an important role in regulating the neurogenesis and differentiation of the nerve cells during the development of the embryonic nervous system. Therefore, the aims of the present study were to investigate the effects of the intermittent hypoxia on the neurogenesis and the expression of Pax6 in the brain of neonatal mice, and hope to explore the mechanisms underlines the effects of intermittent hypoxia on memory.

Materials and methods

Neonatal mice were exposed to the hypobaric hypoxia environment which simulated high altitude of 2 km (16.0% O_2； PaO_2, 60mmHg) and 5 km (10.8% O_2； PaO2, 41mmHg) separately immediately after birth, 4h per day for 4 weeks. Then mice were raised in the normoxic environment for 1 week and 4 weeks, respectively. The control group was raised in the normoxic environment (21% O_2). BrdU (5-Bromo-2-deoxyUridine) was injected into the mice by intraperitioneal injection for 3 consecutive days after hypoxia. In order to observe the newborn neruons whether integrated into the neural circuits, each mouse was given foot electrical stimulation， two hours before sacrifice. Immunofluorence method was employed to detect the expression of the BrdU、Pax6 and c-fos in the brain. Western-blot was performed to quantitative analysis the expression of the Pax6.

Results

1. The effects of the hypoxia on the neurogenesis

(1) Compared with the control group, BrdU positive cells were found localized in the OV, LV, pir, Or, DG, thalamus, and cerebral cortex of the 4-week hypoxia exposed

mice. Moreover, the number of BrdU positive cells in the 2 km hypoxia group was significantly higher than that of in the 5 km hypoxia group.

(2) BrdU, NeuN, and GFAP were co-localized.

(3) BrdU positive cells were still found 4 weeks after the hypoxia exposure.

(4) 4 weeks after the hypoxia exposure, c-fos and the BrdU were co-expression in the NeuN positive cells.

2. The effects of the hypoxia on the expression of the Pax6

(1) In the control mice of 4-week old, Pax6 expressed in a large number of areas, which include OV, VBD, VP, LV, pir, M, aca, Tu, LSI, LSV, BSTLD, MPOM, LPO, 3V, Pe, LH, VMH, BLA, BMA, Aco, BLV, st, LDVL, MGP, VMHDM, alv, Or, PLCo, PMCo, Mtu, BSTAI, MePD, MePV, PH, ns, ec, AStr, BMP, VPM, VM, VPL, DG, LMol, FC, SubG, RPF, ZIV, ZID, and MM.

(2) In the brain of mice exposed to 2 km hypoxia for 4 weeks, Pax6 positive cells were found in the OV, Pir, LV, DG, MTu, BMP, and PLCo. In the brain of mice exposed to 5 km hypoxia for 4 weeks, Pax6 positive cells were found in the OV, pir, Or, Tu, M, VDB, LV, VP, LSI, LSV, MPOM, DM, MCPO, PLCo, MGP, Pe, LH, VMH, BMA, BLA, LDVL, and MePD.

(3) Pax6, NeuN and GFAP were co-localized，and Pax6 ，BrdU were co-expressive in the brain of the hypoxia exposed mice.

(4) The Western-blot analysis showed that ① Compared with the control group, after exposed to 2 km or 5 km hypoxia, or one week after exposed to 2 km or 5 km hypoxia, the number of Pax6 positive cells in the brain of mice was significantly reduced, and ② Expression of the Pax6 in the control group, 2 km hypoxia group and 5 km hypoxia group were increased with the time after the termination of the hypoxia treatment at the point of the termination of the treatment, 1 week after the termination

of the treatment and 4 week termination of the treatment. Compared with the control group which were 8 weeks after birth, the expression of the Pax6 in the 5 km hypoxia group 8 weeks after birth were much higher.

Conclusions

1. Neonatal intermittent hypobaric hypoxia can enhance neurogenesis in the brain of mice, and these new born cells can differentiate into both neurons and astrocytes. Furthermore, these new born cells can survival for a long time, and it may establish loop connection with existing mature neurons

2. Pax6 was also expressed in a mount of nerve cells and astrocytes in the brain after birth, and neonatal hypoxia markedly reduced the expression of Pax6.

3. Pax6 and the BrdU were co-expressive in the NeuN positive cells in the brain of the hypoxia mice, which suggest that Pax6 may play a role in hypoxia induced neurogenesis.

Key words: Intermittent hypoxia; Neurogenesis; Pax6