

铅暴露导致小鼠学习记忆功能障碍及海马蛋白激酶 B 表达降低

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摘要:目的 探讨蛋白激酶 B(PKB)在慢性铅暴露所致小鼠学习记忆功能障碍中的作用。方法 5~6 周龄小鼠交配后,铅暴露组仔鼠通过胎盘、乳汁和饮水饲醋酸铅 2.4,4.8 和 9.6 mmol·L⁻¹,连续 42 d。第 42 天水迷宫实验测平台潜伏期;检测血及脑铅浓度;Sanna 方法检测仔鼠海马 CA1 区长时程增强(LTP)和群峰电位幅值(PS);Western 印迹法检测脑海马总 PKB(t-PKB)及磷酸化 PKB(p-PKB)的表达。结果 与正常对照组相比,铅暴露组小鼠寻找平台时间明显延长($P < 0.05$)。正常对照组血铅为(0.05 ± 0.02)mg·L⁻¹,铅暴露组分别为 0.29 ± 0.06,0.91 ± 0.15 和(1.46 ± 0.37)mg·L⁻¹;正常对照组脑铅为(0.12 ± 0.056)μg·g⁻¹,铅暴露组分别为 2.07 ± 0.55,10.18 ± 1.51 和(14.20 ± 2.63)μg·g⁻¹。学习记忆降低程度与血铅、脑铅浓度成正相关($r = 0.678, r = 0.645, P < 0.01$)。高频刺激后,正常对照组的 PS 幅值明显升高,为刺激前的 1.76 倍,而铅暴露组 PS 幅值下降到刺激前的 85%。与正常对照组比较,暴露铅 4.8 及 9.6 mmol·L⁻¹组,PS 幅值明显下降($P < 0.01$)。铅暴露组的 LTP 诱发成功率亦有所下降。小鼠海马 CA1 区 LTP 损伤程度与血铅、脑铅浓度呈正相关($r = 0.659, r = 0.638, P < 0.01$)。铅暴露组小鼠脑海马 p-PKB 表达均明显降低,并具有浓度效应关系。p-PKB 表达与血脑铅浓度呈负相关($r = -0.840, r = -0.813, P < 0.01$),与学习记忆能力损伤程度呈负相关($r = -0.668, P < 0.01$)。铅对小鼠海马神经元细胞 t-PKB 的表达无影响。**结论** 慢性铅暴露可导致学习记忆功能下降,可能与海马 p-PKB 表达下降有关。

关键词:蛋白激酶 B;铅;海马;学习记忆;长时程增强;群峰电位幅值

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铅暴露会导致儿童学习记忆功能低下,严重影响儿童生长期的智力发育^[1],其机制尚不清楚。本课题组前期研究发现,铅暴露通过影响蛋白激酶 C(protein kinase C, PKC)、细胞外信号调节激酶(extrocellular regulated kinase, ERK)、钙/钙调素依赖性蛋白激酶 II(Ca²⁺/calmodulin dependent protein kinase II, CaMK II)等蛋白激酶的活性,进而导致染铅鼠学习记忆功能下降^[2-8]。PKB/Akt 途径可激活多种底物,调节细胞的存活、分化、增殖和代谢等^[9]。Rodgers 等^[10]报道 PKB/Akt 也参与神经元及胶质细胞的存活、分化和凋亡等。本研究拟观察慢性染铅对小鼠学习记忆行为、长时程增强(long-term potentiation, LTP)及小鼠海马 PKB 表达的影响,进一步探讨铅暴露导致学习记忆功能障碍的机制。

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1 材料与方法

1.1 药物、试剂和仪器

总 PKB 多克隆抗体和磷酸化 PKB Thr473 多克隆抗体(兔抗大鼠、小鼠和人)购自美国 Cell Signaling 公司;辣根过氧化物酶标记的羊抗兔 IgG、羊抗兔 IgG 二抗和 ECL 发光检测液购自北京中杉金桥生物技术有限公司;蛋白预染标志物,美国 Invitrogen 公司;抗 β 肌动蛋白抗体,美国 Santa Cruz; BCA 蛋白浓度测定试剂盒,北京碧云天生物技术有限公司; SDS、丙烯酰胺-甲叉双丙烯酰胺(Acr-Bis)、苯甲基磺酰氟化物、乙二胺四乙酸、抑肽酶、人工脑脊液(ACSF)组分及醋酸铅等试剂均为美国 Sigma 公司产品。

低温高速离心机(Sigma),UP50H 型超声粉碎机(美国 Geprugte Sicherheit 公司),VE-180 型电泳仪(上海天能科技有限公司),DY-II 型转印槽和 DYCP-31DN 型水平电泳仪(北京六一仪器厂),图像分析成像软件(Chem Image 5500 V2.03),ZQP-86 振动切片机、MEZ-8301 微电极放大器、SEN-3301 刺激器、SS-202J 隔离器和 HXD-2000 电信号处理分析软件(北京华翔公司)。

1.2 动物铅暴露方法

昆明系小鼠,体质量 28 ~ 34 g,由中国医科大学实验动物部[许可证号 SCXK(辽)2008-0005]提供。小鼠按每笼雄:雌为1:2自然交配,孕鼠随机分为4组,每组10只:正常对照组,3个铅暴露组为醋酸铅2.4,4.8和9.6 mmol·L⁻¹。每个母鼠最多喂养10只仔鼠。仔鼠出生后通过乳汁染铅,第21天断乳后,仔鼠饮用与母鼠相同的饮用水。正常对照组饮用自来水。于出生后第42天仔鼠进行水迷宫实验,水迷宫实验后,取血样后,断头处死,冰上快速解剖取海马,检测铅含量和测定 LTP。部分海马组织放入液氮中,贮存到 -70℃ 冰箱中,用于 PKB 表达的测定。

1.3 Morris 水迷宫实验^[11]

定位航行实验共进行7d。每天训练分上、下午两段进行,每段训练2次,训练时随机选择一个入水点,将小鼠面向池壁放入水中,观察并记录小鼠寻找并爬上平台所需时间(潜伏期)。4次训练分别从不同的入水点入水,如果小鼠在120s内未找到平台,将其引至平台稳定10s。潜伏期记录为120s,每次训练间隔60s。

1.4 石墨炉原子吸收光谱法^[12]检测血铅和脑铅

水迷宫实验后,以微量注射器经眼眶采静脉血0.5~1.0ml,肝素抗凝。200 μl 静脉血,加2.5ml稀硝酸0.1 mol·L⁻¹,混匀放置过夜,加体积分数0.1的三氯醋酸300 μl,3000 × g 离心15 min,取上清石墨原子吸收法测定血铅含量。取小鼠的两侧海马,称重后加5ml纯硝酸,24h后,加热消化,冷却定容到25ml,石墨原子吸收法测定脑铅含量。

1.5 Sanna 法^[13]检测小鼠海马 CA1 区 LTP

水迷宫实验后,每组取10只仔鼠,快速断头,迅速取出全脑置于0~4℃且用95% O₂和5% CO₂饱和的 ACSF(mmol·L⁻¹: NaCl 124, KCl 4.4, NaH₂PO₄ 1, NaHCO₃ 25, MgSO₄ 1.2, CaCl₂ 2, 葡萄糖 10, pH 7.4)中。氧饱和后,切去小脑和1/3前脑,用胶水将含有海马的脑组织块固定在载物浴碟上,用振动切片机冠状面切厚度为400 μm的脑片。记录电极置于CA1区锥体细胞层。刺激参数为:100 Hz, 100脉冲刺激三串,串间隔10s。

高频刺激(high frequency stimulation, HFS)后,用单脉冲刺激检验诱发的群峰电位幅值(population spike amplitude, PS)变化及变化维持的时间表示,如PS的平均幅值高于或低于基线值的10%以上,并维持30min,被认为统计学有意义,高者定义为LTP。

1.6 Western 印迹法^[14]检测小鼠海马组织 PKB 的表达

取冰冻海马组织,加入适量预冷的细胞裂解液[NaCl 0.1 mmol·L⁻¹, Tris-HCl 0.01 mmol·L⁻¹(pH 7.6), EDTA 1 mmol·L⁻¹(pH 8.0), 抑肽酶 0.1 mg·L⁻¹, PMSF 0.1 mg·L⁻¹], 4℃ 超声粉碎后, 17 000 × g 离心 1 h, 取上清分装。用 BCA 法定量蛋白浓度。上样总蛋白 40 μg, 12% SDS-PAGE 分离蛋白质, 电转移至硝酸纤维素膜, 5% 脱脂牛奶室温封闭 2 h。一抗(1:400), 二抗(1:5000), ECL 试剂显带, X 线片曝光, 显影定影后扫描。利用 ChemiImager 5500 V2.03 图像分析软件对实验结果进行分析, 以目的条带与内参照 β 肌动蛋白的平均吸光度比值表示相对表达水平, 进行半定量分析。

1.7 统计学分析

实验结果数据用 $\bar{x} \pm s$ 表示, 用 SPSS10.0 系统软件对数据进行统计分析。组间比较采用完全随机设计方差分析和重复测量设计方差分析处理, 两两比较采用 *q* 检验, 相关性采用 Pearson 相关分析。

2 结果

2.1 铅暴露对仔鼠平台潜伏期的影响

图1结果显示,与正常对照组相比,铅暴露组第1天寻找平台使用的时间略长于正常对照组,但只有9.6 mmol·L⁻¹组的差异有统计学意义(*P* < 0.05)。从第2天开始,正常对照组所用时间明显缩短,为第1天所用时间的64%;铅暴露组所用的时间虽然也减少,分别为第1天所用时间的64%,

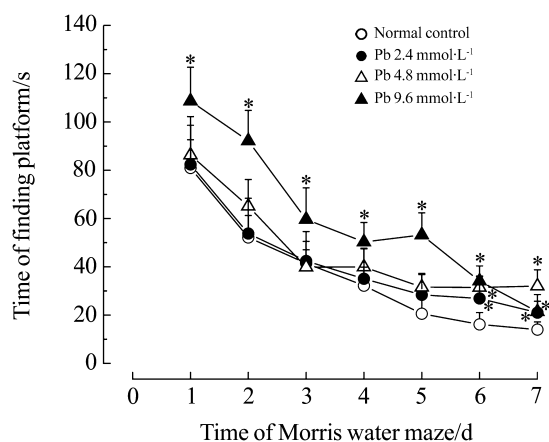


Fig. 1 Effect of lead acetate on time of finding platform.

Young mice were exposed to lead acetate (0, 2.4, 4.8 and 9.6 mmol·L⁻¹) by placenta, milk and drinking water for 42 d consecutively. Morris water maze was determined in postnatal 42 d. $\bar{x} \pm s$, *n* = 10. * *P* < 0.05, compared with corresponding normal control group.

75% 和 86%。第 6 和第 7 天时,铅暴露组所用的时间均高于正常对照组,差异具有统计学意义($P < 0.05$)。提示铅暴露可明显损伤小鼠的空间学习记忆能力。Pearson 相关分析显示,学习记忆能力损伤程度与血铅、脑铅浓度呈正相关($r = 0.678$ 和 0.645 , $P < 0.01$),即随血铅、脑铅浓度升高,空间学习记忆损伤程度越严重。

2.2 铅暴露对仔鼠血铅和脑铅浓度的影响

表 1 结果表明,与正常对照组相比,铅暴露组仔鼠血铅浓度和脑铅浓度显著增加($P < 0.01$),并呈剂量相关性($r = 0.701$ 和 0.678 , $P < 0.01$)。

Tab. 1 Effect of lead acetate exposure on brain and blood lead in mice

Lead acetate/ $\text{mmol} \cdot \text{L}^{-1}$	Blood lead/ $\text{mg} \cdot \text{L}^{-1}$	Brain lead/ $\mu\text{g} \cdot \text{g}^{-1}$
0	0.05 ± 0.02	0.12 ± 0.06
2.4	$0.29 \pm 0.06^{**}$	$2.07 \pm 0.55^{**}$
4.8	$0.91 \pm 0.15^{**}$	$10.18 \pm 1.51^{**}$
9.6	$1.46 \pm 0.37^{**}$	$14.20 \pm 2.63^{**}$

See Fig. 1 for the treatment. $\bar{x} \pm s$, $n = 10$. $^{**} P < 0.01$, compared with $0 \text{ mmol} \cdot \text{L}^{-1}$ group.

2.3 铅暴露对小鼠海马 CA1 区 LTP 的影响

高频刺激前,正常对照组 PS 幅值为 (156 ± 13.7) mV,铅暴露组为 (139.9 ± 13.2) mV,两组无显著性差异。高频刺激后,正常对照组的 PS 幅值明显升高,为刺激前的 1.76 倍,而铅暴露组的 PS 幅值下降到刺激前的 85%,两组在同一时间的 PS 幅值比较有统计学意义(图 2A)。铅暴露 $2.4 \text{ mmol} \cdot \text{L}^{-1}$ 组 PS 幅值与正常对照组比较无显著性差异。如图 2B 所示,与正常对照组比较,铅暴露 4.8 及 $9.6 \text{ mmol} \cdot \text{L}^{-1}$ 组 PS 幅值明显降低($P < 0.01$)。铅暴露组的 LTP 诱发成功率亦有所下降,正常对照组 LTP 诱发成功率为 78% (7/9)。铅暴露组 LTP 诱发成功率为 60% (6/10)。Pearson 相关分析显示,小鼠海马 CA1 区 LTP 损伤程度与血铅、脑铅浓度呈正相关($r = 0.659$ 和 0.638 , $P < 0.01$),即随血铅、脑铅浓度升高,LTP 损伤程度越严重。

2.4 铅暴露对仔鼠海马组织 PKB 表达的影响

图 3 结果显示,与正常对照组相比,铅暴露组 p-PKB 表达显著降低($P < 0.01$),分别降低了 9.7%, 47.2% 和 65.7%; 并与铅浓度呈负相关($r = -0.840$, $P < 0.01$)。铅暴露对总 PKB 蛋白表达无影响。Pearson 相关分析显示,p-PKB 表达与血脑铅浓度呈负相关($r = -0.840$, $r = -0.813$, $P < 0.01$),即血脑铅的浓度越高,p-PKB 表达降低越明

显。p-PKB 表达与学习记忆能力损伤程度呈负相关($r = -0.668$, $P < 0.01$),即 p-PKB 表达降低越明显,学习记忆损伤程度越严重。

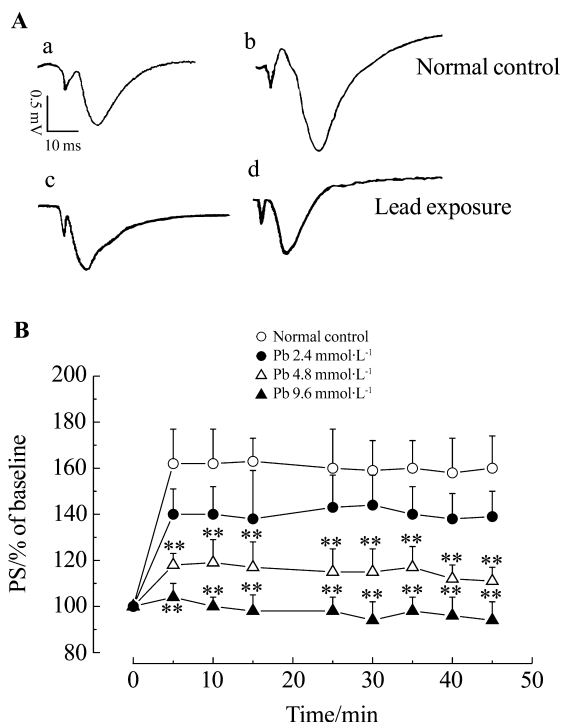


Fig. 2 Effects of lead acetate exposure on long-term potentiation (LTP) in mice. A: examples of original traces of population spike recording from mice of control and chronic lead exposure group in 5 min before high frequency stimulant (HFS) (a, c) and after HFS 20 min (b, d) of normal control and chronic lead exposure groups, respectively. B: defined the average of seven PS before HFS as 100%. $\bar{x} \pm s$, $n = 7$. $^{**} P < 0.01$, compared with corresponding normal control group.

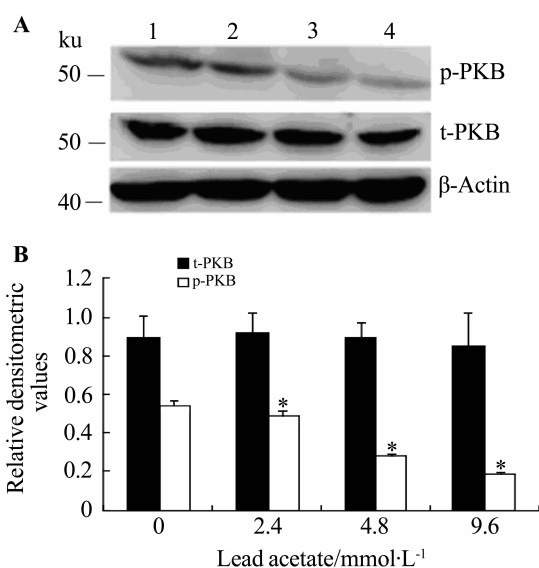


Fig. 3 Effect of lead on protein kinase B (PKB) expression in hippocampus of mice exposed to chronic lead. A: lead acetate $0, 2.4, 4.8$ and $9.6 \text{ mmol} \cdot \text{L}^{-1}$ was given to mice for 6 weeks. B: the semiquantitative result of A. $\bar{x} \pm s$, $n = 4$. $^* P < 0.05$, compared with corresponding control group.

3 讨论

本研究结果提示,慢性铅暴露可明显损伤小鼠的空间学习记忆能力,损伤程度与血铅、脑铅的浓度成正相关;水迷宫结果表明,高浓度染铅可导致空间记忆损伤。有研究证明,基因改变的小鼠在 CA1 区不能诱导 LTP,并显示空间记忆缺失^[15],故选定 CA1 的 LTP 为测定指标。慢性铅暴露鼠海马脑片 LTP 实验证明,铅暴露导致小鼠海马 CA1 区 LTP 异常,高频刺激前正常对照组及铅暴露组的 PS 幅值无明显差异,而高频刺激后,铅暴露组 PS 幅值比正常对照组明显降低。铅暴露组的 LTP 诱发成功率亦有所下降。该结果说明铅暴露可使小鼠 LTP 异常,导致空间记忆损伤。

研究发现,PI3K 激活下游的 PKB/Akt 等参与海马 CA1 区 LTP 的维持期的磷酸化,而与 LTP 的诱导无关^[16]。Western 印迹结果显示,慢性铅暴露对小鼠大脑海马组织 t-PKB 表达水平没有显著性影响,但可降低小鼠大脑海马组织 p-PKB 的表达水平。PKB 磷酸化与血铅浓度呈负相关,与学习记忆能力损伤程度比较呈负相关。该结果说明随染铅浓度增加,PKB 磷酸化量减少,进而导致学习记忆功能低下。因 PKB 的磷酸化在一定程度上代表 PKB 的活性,说明铅是通过影响 PKB 的活性而导致小鼠学习记忆功能下降的。该结果说明 PKB 是铅作用的一个靶点,即铅通过影响中枢神经系统海马神经元细胞信号转导过程中 PKB 的磷酸化,而进一步影响 LTP 的维持,导致学习记忆功能异常。这是铅致学习记忆功能紊乱的原因之一,即慢性铅暴露小鼠脑海马组织 PKB 表达降低与慢性铅暴露小鼠学习记忆功能异常有关。前期研究指出,铅暴露时大脑皮质 PKB 表达也有类似改变^[17],其详细机制有待于进一步探讨。

参考文献:

- [1] Canfield RL, Henderson CR Jr, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter[J]. *N Engl J Med*, 2003, **348**(16):1517-1526.
- [2] Hou WJ, Sun LG, Zhu QW, Wu Z, Liu SY, Xing W. Relationships among plumbum, activity of protein kinase C in the brain tissue of fetal mice and changes in memory function[J]. *Chin J Clin Rehabil* (中国临床康复), 2005, **9**(4):241-243.
- [3] Yang J, Sun LG, Cai K, Zong ZH, Xing W, Liu SY, et al. Effect of acute and chronic lead exposure on CA1-long term potentiation and active extracellular signal-regulated kinase 2 of rat hippocampus[J]. *Chin J Pharmacol Toxicol* (中国药理学与毒理学杂志), 2004, **18**(1):66-70.
- [4] Wen T, Sun LG, Zong ZH, Xing W, Liu SY. Effect of lead exposure on expression of Ca²⁺-calmodulin dependent protein kinase II in mice[J]. *Chin J Pharmacol Toxicol* (中国药理学与毒理学杂志), 2005, **19**(5):393-395.
- [5] Zhang Y, Sun LG, Ye LP, Cao SC, Wang Y. The effect of lead on ERK activity and total of rat primary neural-glia culture [J]. *J Toxicol* (毒理学杂志), 2007, **21**(5):395-398.
- [6] Gao S, Wen F, Sun LG, Gong HZ, Jiang H. Effect of chronic lead exposure on expression of PKC- γ in mice hippocampus[J]. *Chin J Public Health* (中国公共卫生), 2008, **24**(7):793-794.
- [7] Peng B, Wu Z, Zhang CD. Effect of chronic lead exposure on expression of PI3Ks in cortical neuron of mice [J]. *Chin J Public Health* (中国公共卫生), 2010, **26**(2):228-229.
- [8] Peng B, You YY, Gao S, Sun LG. Effect of chronic lead exposure on expression of PI3Ks/PKB pathway in cortical neuron of mice[J]. *J Toxicol* (毒理学杂志), 2012, **26**(2):92-94.
- [9] Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, et al. Mechanism of activation of protein kinase B by insulin and IGF-1 [J]. *EMBO J*, 1996, **15**(23):6541-6551.
- [10] Rodgers EE, Theibert AB. Functions of PI3-kinase in development of the nervous system[J]. *Int J Dev Neurosci*, 2002, **20**(3-5):187-197.
- [11] Selcher JC, Atkins CM, Trzaskos JM, Paylor R, Sweatt JD. A necessity for MAP kinase activation in mammalian spatial learning [J]. *Learn Mem*, 1999, **6**(5):478-490.
- [12] Fernandez FJ. Micromethod for lead determination in whole blood by atomic absorption, with use of the graphite furnace[J]. *Clin Chem*, 1975, **21**(4):558-561.
- [13] Sanna PP, Berton F, Cammalleri M, Tallent MK, Siggins GR, Bloom FE, et al. A role for Src kinase in spontaneous epileptiform activity in the CA3 region of the hippocampus[J]. *Proc Natl Acad Sci USA*, 2000, **97**(15):8653-8657.
- [14] Ye LP, Sun LG, Ren F, Liu P, Zhang Y. Anti-apoptotic signal pathway of bFGF by ERK in ovarian cancer cell line CAO3[J]. *Chin J Anat* (解剖学杂志), 2007, **30**(2):146-149,156.
- [15] Nosten-Bertrand M, Errington ML, Murphy KP, Tokugawa Y, Barboni E, Kozlova E, et al. Normal spatial learning despite regional inhibition of LTP in mice lac-

- king Thy-1[J]. *Nature*, 1996, **379**(6568):826-829.
- [16] Sanna PP, Cammalleri M, Berton F, Simpson C, Lutjens R, Bloom FE, *et al.* Phosphatidylinositol 3-kinase is required for the expression but not for the induction or the maintenance of long-term potentiation in the hippocampal CA1 region[J]. *J Neurosci*, 2002, **22**(9):3359-3365.
- [17] Peng B, Zhang CD, Ren Y, Wu Z. Effect of protein kinase B on the learning and memory functions of mice with chronic lead exposure[J]. *Chin J Neuromed*(中华神经医学杂志), 2009, **8**(4):363-366.

Impairment of learning and memory and decreasing of protein kinase B expression in mice hippocampus induced by lead

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Abstract: **OBJECTIVE** To explore the effect of protein kinase B (PKB) expression on learning and memory in hippocampus neuron of mice exposed to chronic lead. **METHODS** Young mice were exposed to acetic lead 0, 2.4, 4.8 and 9.6 mmol·L⁻¹ by placenta, milk and drinking water for 42 d consecutively, after mice of 5–6 weeks were mated. Morris water maze was determined in postnatal 42 d to observe the capability of spatial learning and memory, blood and brain lead was determined in mice. The population spike (PS) amplitude in CA1 region mice in four groups were alternatively determined by Sanna method. The expression of total PKB (t-PKB) and phosphorylated PKB (p-PKB) determined by Western blotting. **RESULTS** The mean time of finding the platform in lead exposure group was higher than that of the control group ($P < 0.05$). Compared with blood lead in control group (0.05 ± 0.02) mg·L⁻¹; blood lead in lead exposure groups was 0.29 ± 0.06 , 0.91 ± 0.15 and (1.46 ± 0.37) mg·L⁻¹, respectively. Compared with the brain lead in control group was (0.12 ± 0.056) μg·g⁻¹ tissue, brain lead in lead exposure groups was 2.07 ± 0.55 , 10.18 ± 1.51 and (14.20 ± 2.63) μg·g⁻¹, respectively. Chronic acetic lead exposure could damage the capability of spatial learning and memory in mice obviously; the damage level was positive correlated with the concentration of blood and brain lead ($r = 0.678$ and 0.645 , $P < 0.01$). After the application of the high frequency stimulation (HFS), the PS amplitude in control group increased in relation to baseline amplitude to 176%, while in chronic lead exposure group decreased to 85%. PS amplitude of lead 4.8 and 9.6 mmol·L⁻¹ groups was significantly lower than that in the corresponding control group ($P < 0.01$). The incidence of long-term potentiation (LTP) induction of lead exposure group decreased significantly. The damage level of LTP in lead exposure group was positive correlate with the concentration of blood and brain lead ($r = 0.659$, $r = 0.638$, $P < 0.01$). The expression of p-PKB in hippocampus of lead exposure group was decreased significantly dose-dependently. The expression of p-PKB in hippocampus was negatively correlated with the concentrations of blood lead and brain lead ($r = -0.840$, $r = -0.813$, $P < 0.01$), and the capability of spatial learning and memory ($r = -0.668$, $P < 0.01$). The t-PKB protein levels did not change under the same experimental conditions. **CONCLUSION** The chronic acetic lead exposure could depress the function of learning and memory in mice. The decreased expression of p-PKB induced by lead exposure in hippocampus may be the one of the reasons of the damage of learning and memory induced by lead exposure in mice.

Key words: protein kinase B; lead; hippocampus; learning and memory; long-term potentiation; population spike amplitude

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