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# Mechanisms of the protective effect of MK-801 against hypoxic-ischemic brain damage

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**Abstract: Objective** The activation of NMDA receptor plays an important role in the pathophysiological process of hypoxic-ischemic brain damage (HIBD). This paper aims at studying the mechanism of the protective effect of NMDA receptor antagonist MK-801 against HIBD. **Methods** Thirty 7-day-old SD rats were randomly assigned into Normal control, HIBD and HIBD + MK-801 groups (n = 10 each). The rats in the latter two groups were kept in an environment of 8 % $O_2$ after their right common carotid arteries were ligated. The rats in the HIBD + MK-801 group were injected with 0.3 mg/kg of MK-801 intraperitoneally before hypoxia exposure. All rats were sacrificed by decapitation immediately hypoxia and ischemia (HI). The single cell suspension of each hemisphere was prepared and the mitochondrial membrane potential ($\triangle\Psi m$) and intracellular free calcium ($[Ca^{2+}]i$) of the brain cell suspension were measured by flow cytometry and fluorescence spectrophometer respectively. **Results** Compared to the Normal control group, the $\triangle\Psi m$ levels of both hemispheres and the right to left $\triangle\Psi m$ ratio in the HIBD group decreased significantly and the $[Ca^{2+}]i$ level increased significantly ($P < 0.05$ or 0.01); compared to the HIBD group, the $\triangle\Psi m$ level and the right-to-left $\triangle\Psi m$ ratio in the HIBD + MK-801 group increased significantly ($P < 0.05$ or 0.01). There was no difference in the right-to-left $[Ca^{2+}]i$ ratio between the HIBD and the HIBD + MK-801 groups. **Conclusions** MK-801 may protect the neonatal brain from hypoxic-ischemic damage by improving the brain cell mitochondrial function through the "NMDA receptor-other-$\triangle\Psi m$" pathway, but not through the "NMDA receptor-$[Ca^{2+}]i$-$\triangle\Psi m$" pathway.

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**Key words:** MK-801; Hypoxic-ischemic brain damage; Mechanism

## NMDA 受体拮抗剂 MK-801 对缺氧缺血性脑损伤保护作用的机制探讨

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[**摘 要**] **目的** NMDA 型谷氨酸受体的激活在缺氧缺血性脑损伤(HIBD)的病理生理过程中具有重要的作用。该研究探讨 NMDA 型谷氨酸受体拮抗剂 MK-801 对 HIBD 的保护机制。**方法** 30 只 7 日龄 SD 大鼠随机分为正常对照组、HIBD 组和 HIBD + MK-801 组,每组 10 只,后两组大鼠结扎右颈总动脉后暴露于低氧环境制备 HIBD 模型,HIBD + MK-801 组大鼠于低氧处理前腹腔注射 MK-801 0.3 mg/kg。所有大鼠于 HI 后立即断头处死,制备脑单细胞悬液,以流式细胞仪测定脑细胞线粒体跨膜电势($\triangle\Psi m$)、以荧光扫描仪测定脑细胞内游离钙($[Ca^{2+}]i$)水平。**结果** 与正常对照组相比,HIBD 组大鼠双侧脑细胞 $\triangle\Psi m$ 值及右:左 $\triangle\Psi m$ 比值均显著降低,$[Ca^{2+}]i$ 显著升高,差异均有显著性($P < 0.05$或0.01);MK-801 + HIBD 组大鼠脑细胞右:左侧 $\triangle\Psi m$ 比值较 HIBD 组升高,其差异有显著性($P < 0.05$),但两组间脑细胞右:左 $[Ca^{2+}]i$ 的比值无显著性差异($P > 0.05$)。**结论** MK-801 对缺氧缺血性脑损伤的保护作用与其改善脑细胞线粒体功能有关,而与其对 $[Ca^{2+}]i$ 水平的影响关系不大,其保护机制可能不是经过"NMDA 受体-$[Ca^{2+}]i$-$\triangle\Psi m$"途径、而是通过"NMDA 受体-其它-$\triangle\Psi m$"途经发挥作用。

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[**关 键 词**] MK-801;缺氧缺血性脑损伤;保护机制

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Neonatal hypoxic-ischemic brain damage (HIBD) has remained one of the common causes of the neonatal death and long-term neurological deficits. HIBD is directly induced by cerebral hypoxia and ischemia. Mitochondria are key intracellular organelles participating in intracellular ATP metabolism and are related to hypoxia ischemia (HI). The brain cell mitochondrial function is damaged in HIBD[1]. It was reported that mitochondrial dysfunction was closely related to HI-induced NMDA receptor activation[2] and was an early signal in glutamate-induced excitotoxicity[3]. Much research has confirmed that MK-801, a non-competitive NMDA receptors antagonist, can give neuronal protection against HI-induced damage to CNS[4,5]. The objective of this study is to investigate the possible mitochondria-related mechanisms of the protective effect of MK-801 against HIBD.

## Materials and methods

### Animals and grouping

Thirty 7-day-old SD rats were randomly assigned into Normal control, HIBD group and HIBD + MK-801 group (n = 10 each). The rats in the HIBD and HIBD + MK-801 groups were kept in an environment of 8 % $O_2$ and 92 % $N_2$ and with a temperature of 34 ±0.1 ℃ for 2.5 hours after their right common carotid arteries had been ligated. The rats in the HIBD + MK-801 group were injected with 0.3 mg/kg of MK-801 intraperitoneally before hypoxia exposure. All rats were sacrificed by decapitation right after hypoxia.

### Preparation of brain cell suspension[6]

The right and left cerebral hemispheres of the animals were separated (the cerebellum and olfactory bulb were discarded) and placed in the 37 ℃ digest solution with 2 u/ml papain for 30 minutes. The solution was gently triturated, re-suspend and centrifuged three times and the dissociated cells were finally re-suspended at a density of $2 \times 10^6$ cells/ml.

### Mitochondrial membrane potential ($\triangle\Psi m$) measurement

Fluorescent $\triangle\Psi m$ indicator Rhodamine 123 (Rho123) with a final concentration of 1 uM was added into 1 ml of the above cell suspension and was incubated at 37 ℃ for 45 minutes. A Flow cytometry (COULTER EPICS ELITE, Coulter Corperation, USA) was used to measure the fluorescent density of Rho123 (excitation at 488 nm and emission at 525 nm). Ten thousands cells per sample was collected and put into Listmode files and were then was analyzed by WinMDI 2.8 Cytometer Analysis Software (Pruduce University, USA). The mean fluorescence level (MFL) was considered as the $\triangle\Psi m$ value.

### Intracellular free calcium ($[Ca^{2+}]i$) measurement[7]

Fura-2AM with the final concentration of 5 uM was added to 1 ml cell suspension and was then incubated at 37 ℃ for 45 minutes. The $[Ca^{2+}]i$ level was measured using a Hitachi F-4500 Fluorescence Spectrophometer, and the right-to-left $[Ca^{2+}]i$ ratio was calculated.

### Statistical analysis

All data were presented as $\bar{x} \pm s$ and the unpaired 2-tailed student $t$-test was used to analyze the differences.

## Results

### The mean $\triangle\Psi m$ level of each group

Compared to the Normal control group, the $\triangle\Psi m$ levels of both hemispheres and the right-to left $\triangle\Psi m$ ratio in the HIBD group decreased significantly ($P < 0.05$ or 0.01), with the right hemisphere $\triangle\Psi m$ level decreasing by 60 % and the right-to-left $\triangle\Psi m$ ratio decreasing by 24.5 %. Compared to the HIBD group, the $\triangle\Psi m$ level and the right-to-left $\triangle\Psi m$ ratio in the HIBD + MK-801 group increased and the difference was significant ($P < 0.05$ or 0.01) (Table 1).

### The right-to-left $[Ca^{2+}]i$ ratio of each group

The right-to-left $[Ca^{2+}]i$ ratio in the HIBD group was significantly higher than that of the Normal control group (1.41 ±0.16 vs 0.94 ±0.19) ($P < 0.05$), but no difference was found between the HIBD and the HIBD + MK-801 groups.

**Table 1** $\triangle\Psi m$ level and right-to-left $\triangle\Psi m$ ratio in the 3 groups ($n = 10$, $\bar{x} \pm s$)

| Group | $\triangle\Psi m$ (MFL) | | right-to-left $\triangle\Psi m$ ratio |
|---|---|---|---|
| | right hemisphere | left hemisphere | |
| Normal control | 18.93 ±0.74 | 18.21 ±1.26 | 1.06 ±0.05 |
| HIBD group | 7.59 ±0.32[a] | 9.76 ±0.67[b] | 0.80 ±0.06[a] |
| HIBD + MK-801group | 10.83 ±0.41[c] | 10.22 ±0.42 | 1.07 ±0.06[d] |

Note: a vs the Normal control group $P < 0.01$; b vs the Normal control group $P < 0.05$; c vs the HIBD group $P < 0.05$; d vs the HIBD group $P < 0.01$

## Discussion

Normal mitochondrial function is important for the viability of cells and cerebral energy supply. $\triangle\Psi m$ has been regarded as one of the markers of normal mitochondrial function[8]. Based on the metabolic demands of the brain, it is reasonable to speculate that mitochondria are a central target for glutamate neurotoxicity after HI. The NMDA receptors may be activated by HI and result in an increase of calcium influx and accumulation of intracellular free calcium. The changes can cause mitochondrial membrane depolarization (indicated by $\triangle\Psi m$ reduction) under energy depletion. Then the mitochondrial transition pore (MTP) is opened and the depolarization of $\triangle\Psi m$ is aggravated so that cells die[9]. Since NMDA receptors are important membrane receptors in mediating calcium influx, they are considered to play a key role in intracellular calcium disequilibrium and $\triangle\Psi m$ depolarization. Nevertheless, there was still lack of evidence to explain accurately the relationship between the cerebral $\triangle\Psi m$ change and the $[Ca^{2+}]i$ change after NMDA receptors had been antagonized.

Theoretically, it can be deduced that NMDA receptor antagonist may reduce the intracellular free calcium concentration and the depolarization of mitochondrial membrane potential via by blocking the activation of NMDA receptors and ameliorate HI-induced cell damage. In this study, the $\triangle\Psi m$ levels and the right-to-left $\triangle\Psi m$ ratio in the HIBD + MK-801 group were higher than those of the HIBD group, and the right-to-left $[Ca^{2+}]i$ ratio was not different from the HIBD group. It is suggested that MK-801 could improve mitochondrial function by reducing the cerebral $\triangle\Psi m$ depolarization and relieving HIBD. So it can be deduced that the protective effect of MK-801 on HIBD is realized through the "NMDA receptor-other factors-$\triangle\Psi m$" pathway, but not through the "NMDA receptor-$[Ca^{2+}]i$-$\triangle\Psi m$" pathway.

In this study, the increased cerebral $[Ca^{2+}]i$ level and the right-to-left $[Ca^{2+}]i$ ratio after HI was consistent with the pathophysiology of HIBD. But the change of $[Ca^{2+}]i$ level was observed at only one time point after HI (0 h after HI). As the secondary message, calcium can usually make quick responses to various stimuli. It has been reported that glutamate can cause a primary increase and a secondary increase of intracellular free calcium concentrations of suprachiasmatic nucleus neurons within an hour after application of glutamate[10]. It is therefore necessary to study further the change of $[Ca^{2+}]i$ level at the other time points after HI to clarify the accurate relationship between the protective effect of MK-801, and the changes of $\triangle\Psi m$ and $[Ca^{2+}]i$ levels.

In summary, the NMDA receptor antagonist MK-801 can significantly reduce the HI-induced $\triangle\Psi m$ depolarization, but it does not influence the right-to-left $[Ca^{2+}]i$ ratio at an early stage after HI. The neuroprotection of MK-801 on HIBD may be realized through the "NMDA receptor-other factors-$\triangle\Psi m$" pathway, but not through the "NMDA receptor-$[Ca^{2+}]i$-$\triangle\Psi m$" pathway.

**[References]**

[1] Nicholls DG, Budd SL. Neuronal excitotoxicity: the role of mitochondria [J]. Biofactors, 1998, 8(3-4): 287-299.

[2] White RJ and Reynolds IJ. Mitochondrial depolarization in glutamate-stimulated neurons: an early signal specific to excitotoxin exposure [J]. J Neurosci, 1996, 16(18): 5688-5697.

[3] Schinder AF, Olson EC, Spitzer NC, Montal M. Mitochiondrial dysfunction is a primary event in glutamate neurotoxicity [J]. J Neurosci, 1996, 16(19): 6125-6133.

[4] McDonald JW, Silverstein FS, Johnston MV. MK-801 protects the neonatal brain from hypoxic-ischemic damage [J]. European J Pharmacol, 1987, 140(3): 359-361.

[5] Johnston MV, Trescher WH, Ishida A, Nakajima W. Novel treatments after experimental brain injury [J]. Semin Neonatol, 2000, 5(1): 75 - 86.
[6] Maric D, Maric I, Barker JL. Developmental changes in cell calcium homeostasis during neurogenesis of the embryonic cortex [J]. Cereb Cortex, 2000, 10(6): 561 - 573.
[7] Horton JW, White J, Maass D. Protein kinase C inhibition improves ventricular function after thermal trama [J]. J Trauma, 1998, 44(2): 254 - 264.
[8] Ward MW, Rego AC, Frenguelli BG, Nichollas DG. Mitochondrial membrane potential and glutamate excitotoxicity in cultured cerebellar granule cells [J]. J Neurosci, 2000, 20(19): 7208 - 7219.
[9] Li PA, Kristian T, He QP, Siesjo BK. Cyclosporin A enhances survival, ameliorates brain damage, and prevents secondary mitochondrial dysfunction after a 30-minute period of transient cerebral ischemia [J]. Exp Neurol, 2000, 165: 153 - 163.
[10] Quintero J, McMathon DG. Serotin modulates glutamate responses in isolated suprachiasmatic nucleus neurons [J]. J Neurophysiol, 1999, 82(2): 533 - 539.

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·病例报告·

# 先天性外胚层发育不良 1 例报告

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## 1 临床资料

患儿,男,5⁺月,因间断发热 5 月入院。$G_2P_1$,足月顺产,其母孕期无特殊,第 1 胎为人工流产。生后无窒息史,母乳喂养。患儿于生后数天即出现发热,多为午后出现,体温波动在 38 ℃～39 ℃之间,无畏寒、寒颤及抽搐,无腹胀、腹泻等症状,患儿平素汗少,嗜睡。曾经不规则抗感染治疗,疗效不佳。父母均体健,非近亲婚配,家庭中无遗传病史。查体:T 38.3 ℃,P 130 次/min,R 32 次/min,发育、营养稍差,皮肤弹性欠佳,无皮疹,无黄疸及出血点。毛发稀疏,干燥,微黄。心肺腹无异常,外生殖器无异常,生理反射存在,病理征阴性,皮肤痛觉、触觉存在。入院后做胸部 X 片、腹部 B 超及骨髓穿刺无异常发现。血清抗结核抗体、抗 O 及类风湿因子阴性。血沉 43 mm/h。三大常规无异常。血清电解质正常。肝功能正常。血培养阴性及免疫球蛋白无异常。皮肤活检示表皮及真皮经连续切片未见皮肤附件结构。结合病史、体征、实验室检查,诊断为先天性无汗型外胚层发育不良。未经治疗,自动出院。

## 2 讨论

先天性外胚层发育不良又称 christ-siemens 综合征,是一种性联隐性遗传性综合征。由于外胚层的发育不正常所致。可累及皮肤及其附属结构,如牙、眼、指趾甲或波及中枢神经系统。男性患者较多,女性可为携带者。可分为汗分泌过少型(无汗型)和出汗型两种。无汗型为 X 性连锁隐性遗传性疾病,由于汗腺缺乏不能调节体温,常在夏季容易发热和中暑。婴幼儿可发生热性惊厥[1]。患儿可有头发稀软、干燥枯萎,眉毛稀少或 2/3 处无毛,约半数患儿有指趾甲缺陷如甲薄、脆、有嵴或指趾甲发育不良、粗糙、混浊、甲板干燥、松脆或脱落。恒齿数少或缺如。尚有特殊面容。身高偏矮,合并智力低下者约 30 %～50 %。皮肤活检中见表皮角化过度及真皮内缺乏皮肤附件。该病无特殊治疗,主要是帮助患儿适应环境,建立接近正常的生活,婴幼儿夏季给予凉爽环境,预防感染。该病如果处理及时,预防得当,预后尚佳。

[参 考 文 献]

[1] 蔡利璇. 无汗性外胚层发育不良症 1 例 [J]. 中国当代儿科杂志,2001, 3(2): 157.

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