

内侧前额叶皮质区核氧化还原蛋白对卒中后抑郁小鼠抑郁样行为的影响及其机制

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[摘要] **目的:** 探讨内侧前额叶皮质(mPFC)区核氧化还原蛋白(NXN)对小鼠卒中后抑郁(PSD)的影响,并阐明其可能的作用机制。**方法:** 80只C57BL/6小鼠,随机选取42只分为NXN过表达腺相关病毒感染组(AAV-NXN-OE组, $n=21$)和阴性对照腺相关病毒感染组(AAV-NC组, $n=21$),剩余小鼠分为假手术组($n=20$)和PSD组($n=18$),注射NXN过表达腺相关病毒后,将AAV-NXN-OE组和AAV-NC组剩余小鼠分为PSD+AAV-NC组($n=18$)和PSD+AAV-NXN-OE组($n=18$)。术前3周,采用脑立体定位法向小鼠脑组织mPFC区注射NXN过表达腺相关病毒,显微镜下观察病毒在小鼠脑组织mPFC区表达情况,Western blotting法检测小鼠脑组织中NXN蛋白表达水平。采用线栓法建立大脑中动脉闭塞(MCAO)卒中模型,术后1周使用慢性不可预见的中等应激(CUMS)结合孤养法持续干预3周,构建PSD模型小鼠。造模期间监测小鼠体质量变化,造模结束后采用糖水偏好实验、悬尾实验和强迫游泳实验观察各组小鼠抑郁样行为学表现,生化法检测各组小鼠脑组织mPFC区中丙二醛(MDA)和还原型谷胱甘肽(GSH)水平及超氧化物歧化酶(SOD)活性,2',7'-二氯荧光素二乙酸酯(DCFH-DA)荧光探针标记法检测各组小鼠脑组织mPFC区中活性氧(ROS)水平,Western blotting法检测各组小鼠脑组织mPFC区、杏仁核区和海马组织中NXN蛋白表达水平。**结果:** PSD小鼠脑组织mPFC区有大量绿色荧光,表明携带ZsGreen绿色荧光蛋白标签的AAVs病毒在PSD小鼠脑组织mPFC区成功感染并表达。与AAV-NC组比较,AAV-NXN-OE组小鼠脑组织mPFC区中NXN蛋白表达水平明显升高($P<0.05$)。与假手术组比较,PSD组小鼠体质量增长缓慢($P<0.05$),糖水偏好率明显降低($P<0.05$),小鼠在悬尾实验和强迫游泳实验中不动时间均明显增加($P<0.05$);与假手术组比较,PSD+AAV-NC组小鼠糖水偏好率明显降低($P<0.05$),小鼠在悬尾实验和强迫游泳实验中不动时间均明显增加($P<0.05$);与PSD+AAV-NC组比较,PSD+AAV-NXN-OE组小鼠糖水偏好率明显升高($P<0.05$),小鼠在悬尾实验和强迫游泳实验中不动时间均明显减少($P<0.05$)。与假手术组比较,PSD+AAV-NC组小鼠脑组织mPFC区中MDA和ROS水平均明显升高($P<0.05$),GSH水平和SOD活性均明显降低($P<0.05$);与PSD+AAV-NC组比较,PSD+AAV-NXN-OE组小鼠脑组织mPFC区中MDA和ROS水平均明显降低($P<0.05$),GSH水平和SOD活性均明显升高($P<0.05$)。与假手术组比较,PSD组小鼠脑组织mPFC区中NXN蛋白表达水平明显降低($P<0.05$),杏仁核区和海马组织中NXN蛋白表达水平差异无统计学意义($P>0.05$)。**结论:** 小鼠脑组织mPFC区中NXN过表达可改善PSD小鼠抑郁样行为,其作用机制可能与调节氧化还原平衡有关。

[关键词] 卒中后抑郁;核氧化还原蛋白;内侧前额叶皮质;氧化还原平衡

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Effect of nucleoredoxin in medial prefrontal cortex on depression-like behavior in mice with post-stroke depression and its mechanism

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ABSTRACT Objective: To discuss the effect of nucleoredoxin (NXN) in medial prefrontal cortex (mPFC) region of the mice with post-stroke depression (PSD), and to clarify its possible mechanism. **Methods:** A total of 42 mice among 80 C57BL/6 mice were randomly divided into NXN over-expression adeno-associated virus infection group (AAV-NXN-OE group, $n=21$) and negative control adeno-associated virus infection group (AAV-NC group, $n=21$). The remaining mice were divided into sham operation group ($n=20$) and PSD group ($n=18$). After injected with NXN over-expression adeno-associated virus, the remaining mice in AAV-NXN-OE group and AAV-NC group were further divided into PSD+AAV-NC group ($n=18$) and PSD+AAV-NXN-OE group ($n=18$). Three weeks before surgery, NXN over-expression adeno-associated virus was injected into the mPFC region of brain tissue of the mice by stereotaxic method, and the expression of the virus in mPFC region of the mice was observed under microscope. Western blotting method was used to detect the expression levels of NXN protein in brain tissue of the mice in various groups; middle cerebral artery occlusion (MCAO) model was established by thread embolism method, followed one week post-surgery by three weeks of chronic unpredictable moderate stress (CUMS) combined with isolation feeding to construct the PSD mice model. During modeling, the body weight changes of the mice were monitored. After modeling, sucrose preference test, tail suspension test, and forced swim test were used to observe the depressive-like behavioral changes of the mice in various groups; biochemical method was used to detect the levels of malondialdehyde (MDA) and reduced glutathione (GSH), and superoxide dismutase (SOD) activities in mPFC region of brain tissue of the mice in various groups; DCFH-DA fluorescence probe labeling method was used to detect the reactive oxygen species (ROS) levels in mPFC region of brain tissue of the mice in various groups; Western blotting method was used to detect expression levels of NXN protein in mPFC region, amygdala, and hippocampus tissues of the mice in various groups. **Results:** A large amount of green fluorescence was observed in the mPFC region in brain tissue of the PSD mice, indicating successful infection and expression of AAVs virus labeled with ZsGreen green fluorescent protein in mPFC region in brain tissue of the PSD mice. Compared with AAV-NC group, the expression level of NXN protein in mPFC region in brain tissue of the mice in AAV-NXN-OE group was significantly increased ($P<0.05$). Compared with sham operation group, the body weight of the mice in PSD group was increased slowly ($P<0.05$), the sucrose preference rate was significantly decreased ($P<0.05$), and the immobility time of the mice in the tail suspension test and forced swim test was significantly increased ($P<0.05$). Compared with sham operation group, the sucrose preference rate of the mice in PSD+AAV-NC group was significantly decreased ($P<0.05$), and the immobility time in tail suspension test and forced swim test was significantly increased ($P<0.05$). Compared with PSD+AAV-NC group, the sucrose preference rate of the mice in PSD+AAV-NXN-OE group was significantly increased ($P<0.05$), and the immobility time of the mice in tail suspension test and forced swim test was significantly decreased ($P<0.05$). Compared with sham operation group, the MDA and ROS levels in mPFC region in brain tissue of the mice in PSD+AAV-NC group were significantly increased ($P<0.05$), while the GSH level and SOD activity were significantly decreased ($P<0.05$).

Compared with PSD+AAV-NC group, the levels of MDA and ROS in mPFC region in brain tissue of the mice in PSD+AAV-NXN-OE group were significantly decreased ($P<0.05$), while the GSH level and SOD activity were significantly increased ($P<0.05$). Compared with sham operation group, the expression level of NXN protein in the mPFC region of brain tissue of the mice in PSD group was significantly decreased ($P<0.05$), the expression levels of NXN protein in amygdala and hippocampus tissue had no statistically significant difference ($P>0.05$). **Conclusion:** Over-expression of NXN in mPFC region of brain tissue of the mice can improve the depressive-like behavior in the PSD mice, and its mechanism is possibly related to regulating the redox balance.

KEYWORDS Post-stroke depression; Nucleoredoxin; Medial prefrontal cortex; Redox balance

卒中后抑郁 (post-stroke depression, PSD) 是卒中最常见的神经精神并发症之一, 约三分之一的卒中幸存者会出现 PSD^[1]。PSD 患者不仅有抑郁相关的情绪症状, 还伴随自主神经障碍症状, 出现功能独立性下降、认知恢复不良和生活质量下降等情况^[2-3]。我国卒中人群众多, 确诊和潜在的 PSD 患者数量也较为庞大, PSD 带来的高致残率和高死亡率给患者个人、家庭及社会都带来沉重的负担^[4]。然而, PSD 发病机制具有复杂性、多元性和多样性的特点, 给其临床治疗带来较大的挑战, 目前仍缺乏有效且不良反应少的治疗方法。

核氧化还原蛋白 (nucleoredoxin, NXN) 属于硫氧还蛋白家族中的一种氧化还原酶, 通过氧化还原依赖的方式调节不同的信号通路, 从而参与调节多种细胞生物学行为和不同病理机制^[5]。研究^[6]显示: 神经元 NXN 基因敲除小鼠的探索行为减少。NXN 平衡氧化还原功能的丧失会导致感觉神经元发生不良变化, 使神经元 NXN 基因敲除小鼠表现出多发性神经病样的冷回避和热痛超敏反应^[7]。但 NXN 在 PSD 中的作用尚不明确。内侧前额叶皮质 (medial prefrontal cortex, mPFC) 功能损伤是多种精神疾病的基础, 包括抑郁和焦虑等^[8]。本研究检测 NXN 在 PSD 小鼠 mPFC 中的表达水平, 探讨 mPFC 区 NXN 对 PSD 小鼠抑郁样行为的影响, 并阐明相关机制, 旨在为 PSD 治疗新策略的研发提供新的方向。

1 材料与方法

1.1 实验动物、病毒、主要试剂和仪器 80 只 8 周龄 SPF 级雄性 C57BL/6 小鼠, 体质量 22~24 g, 购自武汉大学动物实验中心, 实验动物生产许可证号: SCXK(鄂) 2019-0004。适应性喂养 1 周, 饲养条件为昼夜明暗交替 12 h 循环, 室温 22℃~24℃, 自由进食和饮水。携带 ZsGreen 绿色荧光蛋白标签

的 NXN 过表达腺相关病毒 pHBAAV-CMV-MCS-3flag-EF1-ZsGreen (AAV-NXN-OE) 及其阴性对照慢病毒 (AAV-NC) 由汉恒生物科技 (上海) 有限公司提供。丙二醛 (malondialdehyde, MDA)、超氧化物歧化酶 (superoxide dismutase, SOD)、还原型谷胱甘肽 (reduced glutathione, GSH) 和活性氧 (reactive oxygen species, ROS) 测定试剂盒购自南京建成生物工程研究所, RIPA 裂解液和 BCA 蛋白浓度测定试剂盒购自上海碧云天生物技术有限公司, 兔抗 NXN 单克隆抗体和兔抗 GAPDH 多克隆抗体购自英国 Abcam 公司。荧光显微镜购自日本尼康公司, 冰冻切片机购自上海赛默飞世尔科技有限公司, 荧光酶标仪购自杭州奥盛仪器有限公司, 电泳仪购自北京六一生物科技有限公司。

1.2 脑立体定位注射病毒 随机选取 42 只 C57BL/6 小鼠, 分为 NXN 过表达腺相关病毒感染组 (AAV-NXN-OE 组, $n=21$) 和阴性对照腺相关病毒感染组 (AAV-NC 组, $n=21$), 采用 3% 戊巴比妥钠腹腔注射麻醉小鼠, 浓度为 30 mg·kg⁻¹, 并将小鼠固定在脑立体定位仪上。使用带有 32 号针头的 Hamilton 微量注射器吸取 NXN 过表达腺相关病毒或阴性对照病毒 5×10^{12} v.g·mL⁻¹, 双侧注射入小鼠脑组织 mPFC 区, 单侧注射 400 nL 病毒, 注射位点坐标: 前囟前 +1.90 mm, 中缝左右 ±1.00 mm, 颅骨平面下 -2.10 mm。以 0.1 μL·min⁻¹ 速度进行缓慢注射, 注射结束后, 将针头放置 9 min, 再缓慢抽出。病毒注射 3 周后, 每组取 3 只小鼠 mPFC 组织, 制备冰冻切片, 在显微镜下观察病毒在小鼠脑组织 mPFC 区的表达情况, 并采用 Western blotting 法检测小鼠脑组织中 NXN 蛋白表达水平。

1.3 实验分组和小鼠 PSD 模型构建 随机选取 18 只未感染腺相关病毒的小鼠作为 PSD 组 ($n=18$), 剩下的 20 只未感染腺相关病毒的小鼠作为假手术

组,并将其分为2个亚组,每组10只,再将“1.3”中剩余的AAV-NXN-OE感染小鼠作为PSD+AAV-NXN-OE组($n=18$),剩余AAV-NC感染小鼠作为PSD+AAV-NC组($n=18$)。

除假手术组外,其余各组小鼠均采用线栓法建立大脑中动脉闭塞(middle cerebral artery occlusion, MCAO)模型^[9]:戊巴比妥钠腹腔注射麻醉小鼠,切开颈部中央皮肤,暴露右侧颈总动脉、颈内动脉和颈外动脉。采用7-0手术线于颈外动脉远心端结扎,使用另一根7-0手术线穿过颈外动脉在靠近颈总动脉分叉处打1个活结,于活结上方1.5 mm处的颈外动脉上剪1个小口,将表面涂胶的线栓插入小口,进入颈内动脉盒大脑中动脉,插入线栓约为1 cm。缺血60 min后,拔掉线栓,缝合皮肤。假手术组小鼠仅切开颈部中央皮肤,暴露右侧颈动脉。术后24 h,采用Longa 5分法^[10]对小鼠神经功能进行评分以判断MCAO模型是否成功,选取Longa评分为1~3分的小鼠,于术后第7天开始采用慢性不可预见的中等应激(chronic unpredictable mild stimulus, CUMS)结合孤养法构建PSD小鼠模型^[11]:在孤笼饲养的基础上,每天采用随机和不连续的应激方法刺激小鼠,刺激包括冰水游泳(5 min)、夹尾(15 min)、潮湿垫料(24 h)、禁食(24 h)、禁水(24 h)和昼夜颠倒(12 h/12 h),持续刺激21 d。CUMS期间,每周测定1次小鼠体质量。MCAO术后1周内假手术组小鼠无死亡,PSD组小鼠死亡6只,PSD+AAV-NC组小鼠死亡6只,PSD+AAV-NXN-OE组小鼠死亡5只,而CUMS期间各组均无小鼠死亡。实验结束时,各造模组最终均随机选择10只小鼠进行实验。

1.4 行为学实验观察各组小鼠抑郁样行为 所有小鼠在PSD造模结束后进行行为学检测。①糖水偏好实验:将所有小鼠单笼饲养,实验前1 d进行糖水适应训练,禁食24 h。将分别装有1%蔗糖水和普通饮用水的饮水瓶放于鼠笼中,8 h后取出饮水瓶。记录实验前、后饮水瓶中的液体体积(mL),计算糖水偏好率,糖水偏好率=糖水消耗量(mL)/总液体消耗量(mL) $\times 100\%$ 。②悬尾实验:用胶带缠绕小鼠尾尖1 cm处并倒悬于40 cm \times 40 cm \times 40 cm的悬尾测试箱中,小鼠头部距离测试箱底部约25 cm,小鼠适应2 min后,记录随后4 min累计不动时间,不动时间定义为除正常呼吸外,无其他任何肢体挣扎动作。③强迫游泳实验:将小鼠单独

放入水深15 cm,水温25 $^{\circ}$ C的高30 cm和直径20 cm塑料圆筒中,小鼠适应2 min后,记录随后4 min不动时间。

1.5 生化法检测各组小鼠脑组织 mPFC 区中 MDA 和 GSH 水平及 SOD 活性 行为学检测结束后,采用戊巴比妥钠麻醉假手术组、PSD+AAV-NC组和PSD+AAV-NXN-OE组小鼠后断头取脑,于冰上迅速分离小鼠双侧mPFC组织并放入-80 $^{\circ}$ C环境中冻存。将小鼠脑组织mPFC区匀浆,取上清液。根据生化检测试剂盒说明书操作,分别检测小鼠脑组织mPFC区中MDA和GSH水平及SOD活性。

1.6 2', 7'-二氯荧光素二乙酸酯(2', 7'-dichlorodihydrofluorescein diacetate, DCFH-DA) 荧光探针标记法检测各组小鼠脑组织 mPFC 区中 ROS 水平 取假手术组、PSD+AAV-NC组和PSD+AAV-NXN-OE组小鼠脑组织mPFC区冻存样本,剪碎并进行酶消化处理,加入预冷磷酸盐缓冲液(phosphate buffered saline, PBS)终止消化,500 g离心10 min,收集细胞沉淀。加入DCFH-DA重悬细胞沉淀,室温避光孵育30 min。1 000 g离心10 min,收集细胞沉淀,加入PBS缓冲液重悬,使用荧光酶标仪于激发波长500 nm和发射波长525 nm处检测荧光度值,以假手术组小鼠脑组织mPFC区荧光度值为1,以荧光度值代表各组小鼠ROS水平。

1.7 Western blotting 法检测各组小鼠脑组织不同脑区中 NXN 蛋白表达水平 戊巴比妥钠麻醉小鼠后断头取脑,于冰上迅速分离小鼠脑组织mPFC区、杏仁核区和海马组织。采用RIPA裂解液裂解各组织样本,提取总蛋白。使用BCA蛋白浓度测定试剂盒检测蛋白浓度,SDS-PAGE电泳分离蛋白。湿转法将蛋白转移至PVDF膜,5%脱脂奶粉4 $^{\circ}$ C封闭过夜。加入一抗,NXN抗体(1:1 000)稀释,GAPDH抗体(1:2 000),室温孵育2 h。加入二抗,室温孵育1 h。加入化学发光试剂,曝光显影,分析蛋白条带灰度值,以GAPDH为内参,计算目的蛋白表达水平。目的蛋白表达水平=目的蛋白条带灰度值/内参蛋白条带灰度值。

1.8 统计学分析 采用SPSS 22.0统计软件进行统计学分析。各组小鼠糖水偏好率,悬尾实验不动时间和强迫游泳实验不动时间,小鼠脑组织mPFC区中MDA、GSH和ROS水平及SOD活性,脑组织mPFC区、杏仁核区和海马组织中NXN蛋白表达

水平符合正态分布,以 $\bar{x}\pm s$ 表示,多组间样本均数比较采用单因素方差分析,组间样本均数两两比较采用LSD-*t*检验,2组间样本均数比较采用两独立样本*t*检验。以 $P<0.05$ 为差异有统计学意义。

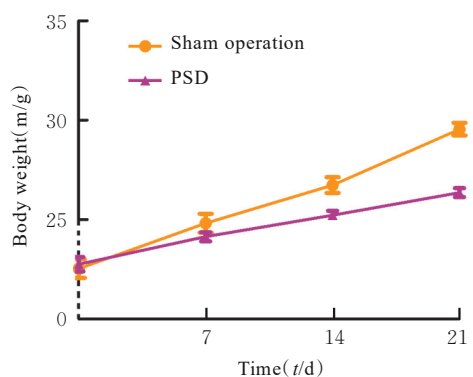
2 结果

2.1 PSD小鼠脑组织 mPFC 区中 NXN 过表达效率

PSD小鼠脑组织 mPFC 区有大量绿色荧光,表明携带ZsGreen绿色荧光蛋白标签的AAVs病毒在PSD小鼠脑组织 mPFC 区成功感染并表达(图1)。与AAV-NC组(0.15 ± 0.03)比较,AAV-NXN-OE组小鼠脑组织 mPFC 区中NXN蛋白表达水平(0.44 ± 0.02)明显升高($P<0.05$)。见图2。

2.2 各组小鼠体质量、糖水偏好率和悬尾实验及强迫游泳实验不动时间与假手术组比较,PSD组小鼠体质量增长缓慢($P<0.05$),糖水偏好率明

显降低($P<0.05$),小鼠在悬尾实验和强迫游泳实验中的不动时间均明显增加($P<0.05$)。见图3和表1。与假手术组比较,PSD+AAV-NC组小鼠糖水偏好率明显降低($P<0.05$),小鼠在悬尾实验和强迫游泳实验中不动时间均明显增加($P<0.05$)。与PSD+AAV-NC组比较,PSD+AAV-NXN-OE组小鼠糖水偏好率明显升高($P<0.05$),小鼠在悬尾实验和强迫游泳实验中不动时间均明显减少($P<0.05$)。见表2。



* $P<0.05$ compared with sham operation group.

图3 CUMS造模期间2组小鼠体质量曲线

Fig. 3 Growth curves of body weights of mice in two groups during CUMS modeling period

表1 2组小鼠糖水偏好率和悬尾实验及强迫游泳实验不动时间

Tab. 1 Sucrose preference rates and immobile time in tail suspension and forced swimming tests of mice in two groups ($n=10, \bar{x}\pm s$)

Group	Sucrose preference rate ($\eta/\%$)	Immobile time (t/s)	
		Tail suspension test	Forced swimming test
Sham operation	74.47 ± 7.46	58.24 ± 7.39	48.07 ± 8.89
PSD	$62.31\pm 6.17^*$	$142.13\pm 10.66^*$	$139.63\pm 9.72^*$

* $P<0.05$ compared with sham operation group.

2.3 各组小鼠脑组织 mPFC 区中 MDA、GSH 和 ROS 水平及 SOD 活性 与假手术组比较,PSD+AAV-NC组小鼠脑组织 mPFC 区中MDA和ROS水平均明显升高($P<0.05$),GSH水平和SOD活性均明显降低($P<0.05$)。与PSD+AAV-NC组比较,PSD+AAV-NXN-OE组小鼠脑组织 mPFC 区中MDA和ROS水平均明显降低($P<0.05$),GSH水平和SOD活性均明显升高($P<0.05$)。见表3。

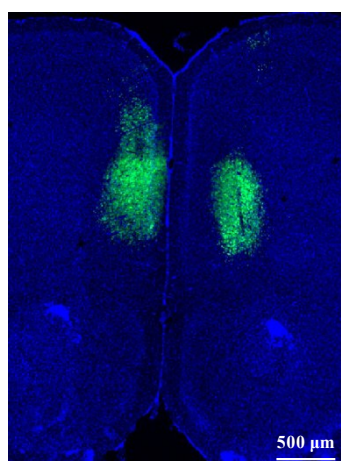
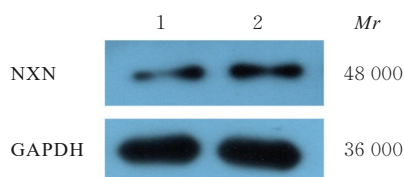


图1 荧光显微镜观察 PSD小鼠脑组织 mPFC 区中 ZsGreen 荧光蛋白表达情况

Fig. 1 Expression of ZsGreen fluorescence protein in mPFC region in brain tissue of PSD mice observed by fluorescence microscope



Lane 1: AAV-NC group; Lane 2: AAV-NXN-OE group.

图2 2组 PSD小鼠脑组织 mPFC 区中 NXN 蛋白表达电泳图

Fig. 2 Electrophoregram of NXN protein in mPFC region in brain tissue of PSD mice in two groups

表2 各组小鼠糖水偏好率和悬尾实验及强迫游泳实验不动时间

Tab. 2 Sucrose preference rates and immobile time in tail suspension and forced swimming tests of mice in various groups

(n=10, $\bar{x} \pm s$)

Group	Sucrose preference rate ($\eta/\%$)	Immobile time (t/s)	
		Tail suspension test	Forced swimming test
Sham operation	75.81 \pm 8.97	57.32 \pm 8.58	47.98 \pm 9.26
PSD+AAV-NC	63.25 \pm 6.51*	141.52 \pm 13.43*	137.96 \pm 12.25*
PSD+AAV-NXN-OE	70.97 \pm 5.82 Δ	122.64 \pm 10.86 Δ	109.95 \pm 8.29 Δ

* $P < 0.05$ compared with sham operation group; $\Delta P < 0.05$ compared with PSD+AAV-NC group.

表3 各组小鼠脑组织 mPFC 区 MDA、GSH 和 ROS 水平及 SOD 活性

Tab. 3 Levels of MDA, GSH, and ROS and activities of SOD in mPFC region in brain tissue of mice in various groups

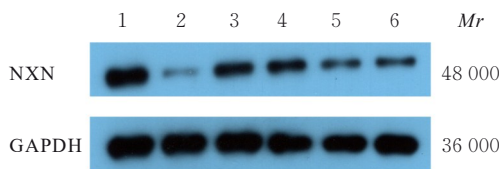
(n=10, $\bar{x} \pm s$)

Group	MDA [$m_B/(\mu\text{mol} \cdot \text{g}^{-1})$]	SOD [$\lambda_B/(\text{U} \cdot \text{mg}^{-1})$]	GSH [$m_B/(\text{mmol} \cdot \text{g}^{-1})$]	ROS
Sham operation	6.24 \pm 1.29	101.28 \pm 10.92	69.85 \pm 3.26	1.00 \pm 0.08
PSD+AAV-NC	16.33 \pm 1.67*	67.89 \pm 5.70*	50.86 \pm 2.92*	1.70 \pm 0.13*
PSD+AAV-NXN-OE	10.98 \pm 2.40 Δ	81.79 \pm 9.08 Δ	63.78 \pm 6.31 Δ	1.43 \pm 0.12 Δ

* $P < 0.05$ compared with sham operation group; $\Delta P < 0.05$ compared with PSD+AAV-NC group.

2.4 2组小鼠脑组织不同脑区 NXN 蛋白表达水平

与假手术组 (mPFC 区: 0.80 \pm 0.09, 杏仁核区: 0.43 \pm 0.06, 海马区: 0.28 \pm 0.06) 比较, PSD 组小鼠脑组织 mPFC 区中 NXN 蛋白表达水平 (0.13 \pm 0.04) 明显降低 ($P < 0.05$), PSD 组小鼠脑组织杏仁核区和海马组织中 NXN 蛋白表达水平 (0.41 \pm 0.08 和 0.27 \pm 0.08) 差异无统计学意义 ($P > 0.05$)。见图 4。



Lane 1-2: mPFC tissue; Lane 3-4: Amygdala tissue; Lane 5-6: Hippocampus tissue; Lane 1, 3, and 5: Sham operation group; Lane 2, 4, and 6: PSD group.

图4 2组小鼠脑组织 mPFC 区、杏仁核区和海马组织中 NXN 蛋白表达电泳图

Fig. 4 Electrophoregram of expressions of NXN protein in mPFC region, amygdala region, and hippocampus tissue in brain tissue of mice in two groups

3 讨论

PSD 发病机制复杂, 包含一些特殊的神经生物学机制。随着神经成像和计算神经科学的发展,

人类对 PSD 发病的神经基础有了更深入的了解^[12]。mPFC 区在多种认知功能中起着重要的调节作用, 研究^[13]表明: mPFC 区是衰老和痴呆时认知能力下降的神经底物。研究^[14]显示: 双酚 A 长期暴露诱导小鼠出现抑郁和焦虑样行为, 其病理机制可能与 mPFC 区锥体神经元形态和功能受损有关。王存强等^[15]通过临床数据分析发现: 海马组织及前额叶皮质神经元代谢异常可能是 PSD 的神经生物学基础。研究^[16-18]显示: 调节 mPFC 区某个基因表达水平, 可通过介导下游信号通路逆转应激诱导的小鼠抑郁相关行为表现。因此, mPFC 区成为抗抑郁药物研发的关键靶向区域之一。本研究采用 MCAO 和 CUMS 结合孤养法构建 PSD 小鼠模型, 小鼠出现抑郁样行为表现, NXN 蛋白在 PSD 小鼠脑组织 mPFC 区中异常低表达, 而其在杏仁核区和海马组织中的表达无明显变化, 提示 mPFC 区中 NXN 表达下调可能参与了 PSD 发病机制。

NXN 作为一种氧化还原调节蛋白, 在氧化还原动态平衡调节过程中发挥重要作用, 但在不同生理和病理环境中的作用机制并不一致。TRAN 等^[6]发现: NXN 可以维持钙调素激酶 2a 的氧化状态和活性, 并支持突触和线粒体蛋白的氧化及线粒体呼吸, 当依赖 NXN 的促氧化功能丧失后, 小鼠的过度动机和活跃减少。研究^[19]发现: 在酒精性肝病体外细胞模型中, NXN 被 ROS 靶向调控, 而 NXN

过表达会与内源性GSH协同作为ROS清除剂,降低细胞氧化应激水平。同时,NXN也被认为是抗氧化系统与免疫反应整合的关键成分。在植物细胞中,NXN通过提高抗氧化能力和诱导热休克蛋白保护细胞免受热应激诱导的氧化损伤和蛋白变性^[20]。因此,NXN在不同生理和病理环境中可能是氧化剂,也可能是还原剂,而NXN平衡氧化还原功能的丧失则可能参与到相关病理机制中。氧化应激在神经精神类疾病的发病机制中发挥关键性作用,也被认为是PSD的致病机制之一^[21]。高水平的氧化应激将产生过量的ROS自由基,导致氧化还原失衡,具有潜在的神经毒性^[22]。研究^[23-24]显示:PSD患者存在氧化应激损伤,以抗氧化应激药物治疗PSD患者取得了较好的改善效果。本研究结果显示:PSD小鼠脑组织mPFC区中NXN蛋白过表达使小鼠抑郁样症状减轻,且脑组织mPFC区中ROS水平降低,抗氧化酶SOD活性和非酶抗氧化剂GSH水平升高,脂质过氧化产物MDA水平降低,即小鼠脑组织mPFC区中氧化应激水平被抑制,提示mPFC区中NXN过表达可降低氧化应激水平,减少PSD小鼠抑郁样行为。

综上所述,NXN在PSD小鼠脑组织mPFC区中低表达,mPFC区中过表达NXN可缓解PSD小鼠抑郁症状,其作用机制可能与调节mPFC区氧化还原动态平衡有关。

利益冲突声明:

所有作者声明不存在利益冲突。

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赵丹参与研究设计、实验指导和论文撰写,史博参与实验操作和论文撰写,魏志玄参与实验操作,崔群建参与数据统计学分析。

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