doi: 10.13241/j.cnki.pmb.2017.04.008

脑源性神经生长因子对齿状回 miR-132 表达及抑制性电流的影响 *

王凤军[1] 李红颖[2] 李 宏[1] 孙悦微[1] 姜杨薇[1] 陈元元[1] 宋婷婷[1]

(1 哈尔滨医科大学附属第四医院 神经内科 黑龙江 哈尔滨 150001;2 哈尔滨市第二医院 神经康复科 黑龙江 哈尔滨 150056)

摘要 目的:探讨脑源性神经生长因子(BDNF)对海马 miR-132 的表达及齿状回颗粒细胞抑制性突触后电流(sIPSCs)的影响,明确 BDNF 对颞叶内侧癫痫(MTLE)发病机制的作用。**方法:**选取哈医大一院神经外科 2008 年 4 月 -2010 年 10 月手术治疗的 MTLE 患者 12 例海马组织。RT-pcr 技术检测 BDNF 孵育后 mir-132 表达,脑片膜片钳技术检测 BDNF 对 sIPSCs 的影响。**结果:**BDNF 升高了颞叶癫痫海马 miRNA-132 的表达(P<0.01),减弱了颗粒细胞 sIPSCs 的频率和幅度(P<0.01)。结论:BDNF 升高了海马 mir-132 的表达,减弱颗粒细胞 sIPSCs 的频率和幅度,可能对 MTLE 的发展有促进作用。

关键词:脑源性神经生长因子;颞叶内侧癫痫;miRNA-132;自发性抑制性突触后电流

中图分类号:R742.1 **文献标识码:**A **文章编号:**1673-6273(2017)04-633-03

Effect of BDNF on The Levels of miRNA-132 and sIPSCs in the Dentate Gyrus of MTLE*

WANG Feng-jun[1], LI Hong-ying[2], LI Hong[1], SUN Yue-wei[1], JIANG Yang-wei[1], CHEN Yuan-yuan[1], SONG Ting-ting[1]

(1 Department of Neurology,The Fourth Hospital of Harbin Medical University, Harbin, Heilongjiang, 150001, China;

2 Department of Rehabilitation,The Second Hospital of Harbin , Harbin, Heilongjiang, 150056, China)

ABSTRACT Objective: To study the effect of brain derived neurotrophic factor (BDNF) on the levels of miRNA-132 and sIPSCs in the dentate gyrus of patients with MTLE. **Methods:** The study was performed from April 2008 to October 2010. Surgically removed specimens were collected from the patients with MTLE. All patients gave written informed consent for research use of the biopsy materials. Surgically resected hippocampal were collected and immediately immersed in oxygenated ice-cold SACSF. The expression of mIR-132 by RT-PCR and sIPSCs by patch-clamp. **Results:** The expression of miR-132 was significantly increased in Dentate Gyrus of MTLE patients after BDNF perfusion was significantly higher than that before perfusion P<0.01). The frequency and amplitude of sIPSC were obviously lower in Dentate Gyrus of MTLE patients after BDNF perfusion was significantly higher than that before perfusion (P<0.01). **Conclusions:** BDNF increased the expression of miR-132 and inhibited the frequency and amplitude of sIPSCs of granule cells in the hippocampus of MTLE patients after operation, which could contribute to the development of MTLE.

Key words: BDNF; MTLE; miRNA-132; sIpscs

Chinese Library Classification(CLC): R742.1 Document code: A

Article ID: 1673-6273(2017)04-633-03

前言

颞叶内侧癫痫(mesial temporal lobe epilepsy,MTLE)是较为常见的难治性癫痫的类型,海马在其发病机制中起到了主要的作用[1],其特征性的病理改变为神经元的丢失和苔藓纤维发芽 (mossy fiber sprouting)[2,3]。脑源性神经营养因子 (brain derived neurotrophic factor,BDNF)是一种促进神经元生长、神经再生及维持神经元存活和功能的营养因子, 与细胞表面 TrkB 受体结合影响多条胞内信号转导路径及突触传递[4,5],其异常表达与多种神经系统疾病发病机制密切相关。大量研究表明癫痫动物模型海马神经元 BDNF 表达会明显升高[6,7]。有研究者报道海马神经元的凋亡导致 BDNF 合成分泌过多从而促进癫痫的发生[8,9]。内源性 BDNF 的上调的同时会加重抽搐导致的神经元损伤,BDNF 相应的减少会减弱抽搐发作所致神经元的损伤,说明 BDNF 的增加或许会导致神经元的损伤[10]。miRNA 是一种类似于 siRNA 的分子,在物种进化过程相当保守,对细胞生长和发育过程中起多种调节作用。研究表明某些 miRNA 能明显的抑制癫痫发作,如 miRNA-124[11,12]、miRNA-23b-3p 等[13]。miRNA-132 在脑组织中表达丰富, 有研究表明其在癫痫海马中的表达明显增高,可能参与癫痫的发生[14,15]。BDNF 与 miRNA 在神经系统的发病机制中可能有交互作用[16,17]。因此,本研究旨在探讨 BDNF 对海马 miR-132 的表达及齿状回颗粒细胞抑制性突触后电流(sIPSCs)的影响,进而明确 BDNF 在 MTLE 发病中的作用。

1 材料与方法

1.1 试剂与材料

脑源性神经生长因子(BDNF)、甲碘荷包牡丹碱(BMI)、生

* 基金项目:黑龙江省卫生厅科研基金项目(2013101)

作者简介:王凤军(1978-),男,博士,副主任医师,主要研究方向:脑血管病,电话:13845072274,E-mail:fengjun19791209@163.com

(收稿日期:2016-09-30 接受日期:2016-11-02)

物胞素(Biocytin)、异去甲槟榔次碱(Isoguvacine hydrochloride)、木防己苦毒素 (PTX)、多聚甲醛(paraformaldehyde)、NaCl、$CaCl_2$、NaH_2PO_4、$NaHCO_3$、KCl、$MgSO_4 \cdot 7H_2O$、KOH、EGTA、$MgCl_2$、HEPES、dextrose、K2-ATP、sucrose 均购自 sigma 公司(St Louis, MO, USA)、超纯水、蒸馏水、组织胶水、PCR 引物(上海生物工程技术有限公司)。

1.2 方法

1.2.1 RT-PCR BDNF (100 ng/mL) 应用 1 小时前后检测 mir-132 的表达。按照试剂盒说明书,循环阈值表达结果,均值加减 SD,各组间差异以△△CT=△CT(目的基因)-△CT(actin),应用 $2^{-\triangle\triangle CT}$ 方法来检测 miRNA 的相对表达。BDNF(100 ng/mL)应用 1 小时前后分别为 Control 组及 BDNF 组。

1.2.2 Whole-cell 记录及 BDNF(100 ng/mL)灌流 齿状回颗粒细胞电压钳制在 -20 mV,APⅤ(50 μm)与 CNQX(20 μm)加入到灌流液中用以阻断兴奋性电流。记录 sIPSCs 稳定后,灌流液中加入 BDNF(100 ng/mL)、BDNF(100 ng/mL)应用前后分别为 Control 组及 BDNF 组。

1.3 统计学分析

数据应用 SPSS13.0 统计软件包分析,均数士标准差($\bar{X}\pm$SD)表示,两样本均数采用 t 检验,以 P<0.05 认为差异有统计学意义。

2 结果

2.1 BDNF 增高海马 mir-132 的表达

12 例 MTLE 患者术后海马组织经 BDNF(100 ng/mL)灌流 1 小时后 mir-132 表达较灌流前明显增加, 差异有统计学意义(P<0.01)。

2.2 BDNF 抑制自发性抑制性突触后电流的频率和幅度

齿状回颗粒细胞电压钳制在 -20 mV,形成记录稳定后,APⅤ(50 μm)和 CNQX(20 μm)加入到灌流液中阻断兴奋性电流。灌流液内加入 BDNF (100ng/ml)。记录 6 个颗粒细胞测量 sIPSCs 的动力学特性。常规监测输入阻抗(10-25MΩ),以输入阻抗变化不超过 15%为标准。结果表明 BDNF 应用前后比较 sIPSCs 幅度明显减小,差异有统计学意义(P<0.01);BDNF 组与 Control 组比较 sIPSCs 频率明显减弱,差异有统计学意义(P<0.01)。Washout 组分别与 Control 组比较, 差异无统计学意义(P>0.05)。

3 讨论

IPSC 主要是由 GABA 所诱导。动物与人类的癫痫脑组织中都有不同程度 GABA 能神经元的减少[18,19]。GABA 神经元的轴突发芽和和神经环路重组都可能促进癫痫的发生[20]。戊四氮

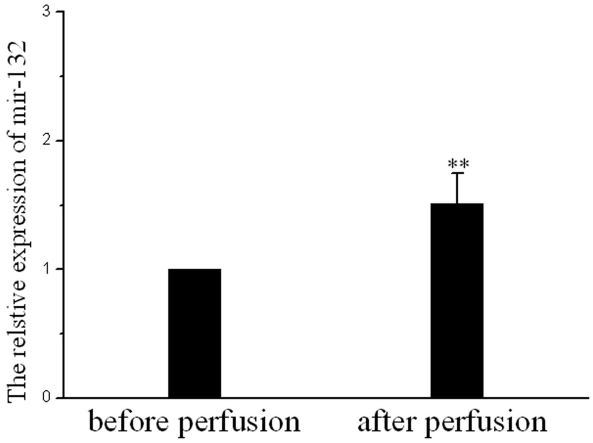

图 1 BDNF 增高了 MTLE 患者术后海马组织 miR-132 的表达

Fig. 1 BDNF increased the expression of miR-132 in the hippocampus of MTLE patients after operation

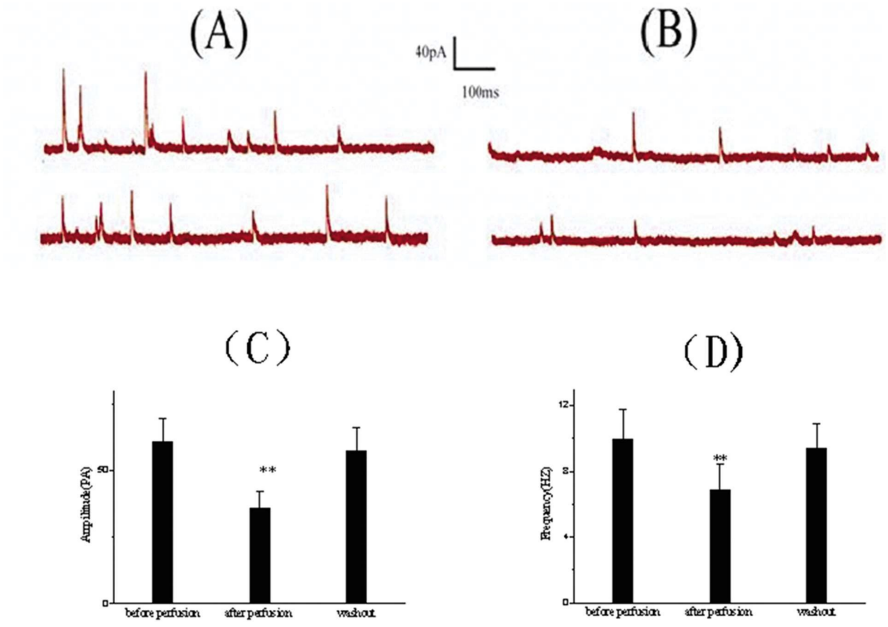

图 2 BDNF 抑制 MTLE 患者术后海马齿状回颗粒细胞自发性抑制性突触后电流的频率和幅度

Fig. 2 BDNF inhibited the frequency and amplitude of sIPSCs of granule cells of MTLE patients after operation

注:与灌流前比较,** P<0.01;与 Washout 比较,** P<0.01。

Note: compared with before perfusion, ** P<0.01; compared with washout, ** P<0.01.

作为 GABAA 的受体阻滞剂可以诱导癫痫模型[21]，通过下调 BDNF 信号可阻止 GABA 神经元的凋亡及抑制海马 CA1 神经元的激活[22]。GABA 为中枢神经系统最重要的抑制性递质，兴奋性递质与抑制性递质失衡为癫痫发病的本质，抑制神经元的兴奋性也是癫痫治疗的基本原理。本研究结果显示 BDNF 可迅速降低 sIPSCs 的频率和幅度，说明 BDNF 降低了抑制性的作用，相对的增强了兴奋性进而对癫痫的发病可能起到促进作用。虽然此结果未能完全阐明该作用是通过突触前还是突触后实现的，但 BDNF 的这种对神经网络的兴奋性作用是肯定的。

BDNF 作为近年癫痫研究的热点因素，对癫痫发病起到的作用不尽相同。多数学者认为 BDNF 对癫痫的发生有促进作用[23,24]。但具体机制并不明确，而且多数研究对象是动物。研究表明 miRNA-132 通过影响神经元树突及细胞的凋亡、兴奋性对癫痫的发病起到促进作用，干预 miRNA 被视为未来抗癫痫的发展方向。本研究结果表明 BDNF 明显增加人类 miRNA-132 的表达，进而说明其可能与癫痫发病的关系。

综上所述，BDNF 对 sIPSCs 及 miRNA-132 的作用都可能促进了癫痫疾病的发生，但 BDNF 这种兴奋性作用与 miRNA-132 表达上调是否存在相互关系还不能确定，我们下一步将继续明确此机制。

参 考 文 献(References)

[1] Elkommos S, Weber B, Niehusmann P, et al. Hippocampal internal architecture and postoperative seizure outcome in temporal lobe epilepsy due to hippocampal sclerosis[J]. Seizure, 2016, 35(2): 65-71

[2] Isgor C, Pare C, McDole B, et al. Expansion of the dentate mossy fiber-CA3 projection in the brain-derived neurotrophic factor-enriched mouse hippocampus[J]. Neuroscience, 2015, 288(3): 10-23

[3] Botterill JJ, Brymer KJ, Caruncho HJ, et al. Aberrant hippocampal neurogenesis after limbic kindling: Relationship to BDNF and hippocampal-dependent memory [J]. Epilepsy Behav, 2015, 47(7): 83-92

[4] Liu X, Liu J, Liu J, et al. BDNF-TrkB signaling pathway is involved in pentylenetetrazole-evoked progression of epileptiform activity in hippocampal neurons in anesthetized rats[J]. Neurosci Bull, 2013, 29 (5): 565-575

[5] Semaan S, Wu J, Gan Y, et al. Hyperactivation of BDNF-TrkB signaling cascades in human hypothalamic hamartoma (HH): a potential mechanism contributing to epileptogenesis [J]. CNS Neurosci Ther, 2015, 21(2): 164-172

[6] Sha'ari HM , Haerian BS, Baum L, et al. Association of BDNF Polymorphisms with the Risk of Epilepsy: a Multicenter Study [J]. Mol Neurobiol, 2016, 53(5): 2869-2877

[7] Scharfman HE, Goodman JH, Sollas AL, et al. Spontaneous limbic seizures after intrahippocampal infusion of brain-derived neurotrophic factor[J]. Exp Neurol, 2002, 174(2): 201-214

[8] Zheng F, Wang H. NMDA-mediated and self-induced bdnf exon IV transcriptions are differentially regulated in cultured cortical neurons [J]. Neurochem Int, 2009, 54(5-6): 385-392

[9] Kolarow R, Brigadski T, Lessmann V. Postsynaptic secretion of BDNF and NT-3 from hippocampal neurons depends on calcium calmodulin kinase II signaling and proceeds via delayed fusion pore opening[J]. Neurosci, 2007 , 27(27): 10350-10364

[10] Adachi N , Numakawa T, Richards M, et al . New insight in expression, transport, and secretion of brain-derived neurotrophic factor: Implications in brain-related diseases [J]. World J Biol Chem, 2014, 5(4): 409-428

[11] Wang WX , Wang L , Chen, et al . The Microrna Mir-124 Suppresses Seizure Activity and Regulates Creb1 Activity [J] . Expert Rev Mol Med, 2016, 18(3): e4

[12] Brennan GP, Dey D, Chen Y, et al. Dual and Opposing Roles of MicroRNA-124 in Epilepsy Are Mediated through Inflammatory and NRSF-Dependent Gene Networks [J]. Cell reports, 2016, 14 (10): 2402-2412

[13] Zhan L, Yao Y, Fu H, et al. Protective role of miR-23b-3p in kainic acid-induced seizure[J]. Neuroreport, 2016, 27(10): 764-768

[14] Guo J H, Wang Q, Wang Y, et al. Expression of P-Creb and Activity-Dependent Mir-132 in Temporal Lobe Epilepsy [J]. Int J Clin Exp Med, 2014, 7(5): 1297-1306

[15] Huang Y, J Guo, Q Wang, et al. Microrna-132 Silencing Decreases the Spontaneous Recurrent Seizures [J]. Int J Clin Exp Med, 2014, 7 (7): 1639-1649

[16] Jimenez-Mateos E M, I Bray, A Sanz-Rodriguez, et al. Mirna Expression Profile after Status Epilepticus and Hippocampal Neuroprotection by Targeting Mir-132[J]. Am J Pathol, 2011, 179(5): 2519-2532

[17] HJ You , JH Park, H Pareja-Galeano, et al. Targeting MicroRNAs Involved in the BDNF Signaling Impairment in Neurodegenerative Diseases[J]. Neuromolecular Med , 2016, 10(16): 8407-8409

[18] Hang P, Sun C, Guo J, et al . Bdnf-mediates down-regulation of microrna-195 inhibits ischemic cardiac apoptosis in rats [J]. International journal of biological sciences, 2016, 12(8): 979-989

[19] Kong S, Cheng Z, Liu J, et al. Downregulated GABA and BDNF-TrkB pathway in chronic cyclothiazide seizure model [J]. Neural Plast, 2014, 3(10): 146

[20] Schwarzer C, Williamson JM, Lothman, EW, et al. Somatostatin, neuropeptide Y, neurokinin B and cholecystokinin immunoreactivity in two chronic models of temporal lobe epilepsy [J]. Neuroscience, 1995, 69(3): 831-845

[21] Wittner L, Eross L, Szabo Z , et al . Synaptic reorganization of calbindin-positive neurons in the human hippocampal CA1 region in temporal lobe epilepsy[J]. Neuroscience, 2002, 115(3): 961-978

[22] Lim BV, Shin MS, Lee JM ,et al . Treadmill exercise prevents GABAergic neuronal loss with suppression of neuronal activation in the pilocarpine-induced epileptic rats [J]. Exerc Rehabil, 2015, 11(2): 80-86

[23] Laura A ,Ewell , Liang Liang , et al . Brain State Is a Major Factor in Preseizure Hippocampal Network Activity and Influences Success of Seizure Intervention[J]. Neurosci,2015, 35(47): 15635-15648

[24] Yang T, Zhou D, Stefan H, et al.Why mesial temporal lobe epilepsy with hippocampal sclerosis is progressive: uncontrolled inflammation drives disease progression [J]. Journal of the Neurological Sciences, 2010, 296(12): 1-6