TRPV2激活对缺氧再灌注时体外培养星形胶质细胞神经生长因子释放的影响

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摘要 神经生长因子对脑缺血后神经元的存活有重要意义。该研究观察了TRPV2激活剂2APB对体外缺血再灌注模型中原代培养大鼠大脑皮层星形胶质细胞神经生长因子释放的影响。将原代培养大鼠大脑皮层星形胶质细胞分为2APB组(0.5 mmol/L)和对照组(不含2APB), 在糖氧剥夺情况下培养2 h, 然后恢复正常全培养基复氧培养48 h。用Western blot检测星形胶质细胞神经生长因子的表达水平; 用ELISA检测星形胶质细胞条件培养液中神经生长因子的含量。结果表明, 0.5 mmol/L 2APB可以诱导正常情况下及糖氧剥夺再灌注情况下体外培养星形胶质细胞NGF的合成和释放(P<0.01)。此外, JNK阻滞剂可抑制糖氧剥夺再灌注情况下2APB诱导的星形胶质细胞神经生长因子的释放。综上, TRPV2激活可以影响糖氧剥夺再灌注情况下体外培养星形胶质细胞神经生长因子的合成和释放。TRPV2有可能成为脑缺血再灌注后的潜在治疗靶点。

关键词 星形胶质细胞; 糖氧剥夺/再灌注; TRPV2; 神经生长因子

TRPV2 Activation Enhances the Expression of Nerve Growth Factor in Primary Cultured Astrocytes Under Oxygen-glucose Deprivation/Reoxygenation

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Abstract Nerve growth factor (NGF) is essential for neuronal growth and survival. We had addressed the effect of 2APB, an activator of TRPV2, on the NGF expression in astrocytes exposed to oxygen-glucose deprivation/reoxygenation. Primary cultured cortical astrocytes stimulated with or without 2APB (0.5 mmol/L) were treated with glucose-deprivation media under oxygen-glucose deprivation for 2 h and then incubated under normal culture condition for 48 h. The levels of NGF protein in astrocytes were analyzed by Western blot and NGF protein levels in astrocyte-conditioned media were measured by enzyme-linked immunosorbent assay (ELISA). 2APB increased the expressions of NGF protein in astrocytes under normal and reoxygenation condition. After 48 h reoxygenation,

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0.5 mmol/L of 2APB enhanced the levels of NGF protein in astrocyte-conditioned media by 1.88-fold of the control. An inhibitor of c-Jun N-terminal kinase (JNK) suppressed the effect of 2APB on the NGF expression. We concluded that 2APB enhanced, via the JNK pathway, NGF expression in astrocytes. TRPV2 activation might have therapeutic potential for brain injury in ischemia/reperfusion.

Key words astrocyte; oxygen-glucose deprivation/reoxygenation; TRPV2; nerve growth factor

神经生长因子(nerve growth factor, NGF)主要由星形胶质细胞分泌[1], 对神经元的存活、生长及功能维持都发挥了重要的作用[2-3]。脑缺血时, 星形胶质细胞活化增殖, NGF表达上调, 对神经元发挥了一定保护作用[4]。

瞬时感受器电位离子通道香草素受体2(transient receptor potential vanilloid 2, TRPV2)是瞬时感受器电位离子通道家族(transient receptor potential, TRP)香草素受体亚家族(transient receptor potential vanilloid, TRPV)的一员, 主要表达于星形胶质细胞, 对Ca^{2+}有高度通透性[5-6]。而脑缺血时, 星形胶质细胞内增高的Ca^{2+}浓度可以诱发星形胶质细胞释放神经营养因子及炎性介质等物质, 决定神经元的存亡与血管神经的再生[7-8]。本研究主要通过调控体外培养星形胶质细胞TRPV2, 观察其对脑缺血时星形胶质细胞NGF释放的影响, 探讨TRPV2与脑缺血时星形胶质细胞分泌功能的关系。

1 材料与方法

1.1 材料与试剂

初生SD乳鼠30只, 由华中科技大学同济医学院动物实验中心提供。DMEM/F12培养基、胎牛血清、胰蛋白酶购自美国Hyclone公司; TRPV2激活剂2APB和多聚赖氨酸购自美国Sigma公司(St. Louis, MO, USA); NGF ELISA试剂盒购自美国Promega公司(Madison, WI, USA); p38 MAPK阻滞剂SB203580购自Enzo Life Science公司(Farmingdale, NY, USA); MEK1/2阻滞剂U0126和JNK阻滞剂SP600125购自Millipore公司(Billerica, MA, USA); 其余抗体均购自Cell Signal Technology公司(Beverley, MA, USA)。

1.2 大鼠乳鼠大脑皮层星形胶质细胞原代培养

将Kliot等[9]的方法改进后行大鼠乳鼠大脑皮层星形胶质细胞原代培养。无菌条件下取新生2~3 d内大鼠大脑, DMEM/F12漂洗3遍, 剔除软脑膜, 取大脑皮层, 用眼科剪剪碎后, 巴氏管吹打, 直至分散为细胞悬液, 经直径200目的筛网过滤; 1 000 r/min离心8 min, 去上清, 全培养基(DMEM/F12培养液+20%胎牛血清)重悬。置于37 ℃、95% O_2、5% CO_2培养箱中培养; 2~3 d换一次液。细胞生长至融合时按1:(2~3)传代至新培养瓶培养, 或按3×10^5的密度接种于多聚赖氨酸包被的盖玻片。培养的星形胶质细胞按照形态特点和免疫荧光染色进行鉴定。实验所用细胞为传2代细胞。

1.3 缺血再灌注模型

本研究通过糖氧剥夺/再灌注(oxygen-glucose deprivation/reoxygenation, OGD/Reoxygenation)的方法建立体外脑缺血再灌注模型。具体如下: 将细胞随机分为4组: Normoxia组、Normoxia+2APB组、OGD/Reoxygenation组(简称为: OGD/Reoxy组)和OGD/Reoxygenaton+2APB组(简称为: OGD/Reoxy+2APB组)。Normoxia组及Normoxia+2APB组分别用含糖EBSS缓冲液(含6.8 g/L NaCl、0.4 g/L KCl、0.11 g/L NaH_2PO_4、0.2 g/L $CaCl_2$、2.2 g/L $NaHCO_3$、0.1 g/L $MgSO_4\cdot2H_2O$、1 g/L葡萄糖, pH7.4)清洗体外培养星形胶质细胞2次后, 加含糖EBSS缓冲液; 其中, Normoxia+2APB组同时加入0.5 mmol/L 2APB; 将上述2组细胞置于含5% CO_2的培养箱(Thermo Scientific)培养2 h, 然后将培养基更换为完全培养基(DMEM/F12+20% FBS); 其中, Normoxia+2APB组同时加入0.5 mmol/L 2APB; 并置于含5% CO_2的培养箱中继续培养相应再灌注时间后, 提取上清。

对于OGD/Reoxy组及OGD/Reoxy+2APB组, 分别用无糖EBSS缓冲液(含6.8 g/L NaCl、0.4 g/L KCl、0.11 g/L NaH_2PO_4、0.2 g/L $CaCl_2$、2.2 g/L $NaHCO_3$、0.1 g/L $MgSO_4\cdot2H_2O$, pH7.4)清洗体外培养星形胶质细胞2次后, 加无糖EBSS缓冲液; 其中, OGD/Reoxy+2APB组同时加入0.5 mmol/L 2APB; 将上述两组细胞置于含94% N_2、1% O_2及5% CO_2的缺氧培养箱(Thermo Scientific三气培养箱)培养2 h, 然后将无糖EBSS缓冲液更换为正常全培养基(DMEM\F12+20% FBS); 其中, OGD/Reoxy+2APB组同时加入0.5 mmol/L 2APB; 并置于含5% CO_2的正常培养箱

继续培养相应再灌注时间后, 提取上清。

1.4 细胞活力检测

本研究通过MTT染色、台盼蓝染色及观察星形胶质细胞形态变化监测体外培养星形胶质细胞细胞活力。星形胶质细胞形态通过GFAP染色观察, 台盼蓝染色按照Patterson的方法进行[10]。MTT染色方法如下: 首先, 将处于对数生长期的细胞经0.25%胰蛋白酶消化后调整细胞浓度至1×10^4/mL, 接种至96孔培养板, 每孔160 μL; 接着用全培养基(DMEM/F12培养液+20% FBS)培养约18~24 h, 待细胞贴壁生长良好后开始MTT检测; 检测中, 实验分4组: Normoxia组、Normoxia+2APB组、OGD/Reoxy组和OGD/Reoxy+2APB组; 每组10孔; 分别在OGD再灌注后0, 6, 12, 24, 48 h, 每孔加入40 μL MTT液; 然后37 °C、95% O_2、5% CO_2培养箱中孵育2 h; 吸走MTT液, 每孔加入150 μL DMSO呈色; 轻微震荡后以空白调零组调零, 用酶标仪在460 nm波长处测定各孔的吸光度值(*D*值)。

1.5 ELISA检测NGF的分泌

将星形胶质细胞条件培养液离心10 min, 取上清, 置于–80 °C冰箱保存。根据试剂盒的检测方法测量条件培养液中所含的NGF。

1.6 Western blot检测NGF的合成

根据Ramsay等[36]的方法用Western blot检测星形胶质细胞NGF蛋白及pJNK蛋白的表达。将处于对数生长期的细胞经0.25%胰蛋白酶消化后按3×10^5/mL的密度接种至10 cm细胞培养皿; 全培养基(DMEM/F12培养液+20% FBS)培养约12~18 h, 待细胞贴壁生长良好, 大约铺满50%培养孔后开始实验; 实验分为Normoxia组、Normoxia+2APB组、OGD/Reoxy组 和OGD/Reoxy+2APB组; 再灌注相应时间后提蛋白。将等量的蛋白标本加入SDS-PAGE凝胶的加样孔, 电泳90 min; 然后电转至NC膜; 封闭液封闭2 h后; 加入相应一抗, 4 °C孵育过夜; TBST漂洗3遍后, 加入1:6 000稀释的Odyssey二抗, 避光孵育2 h; 用Odyssey IR显像系统检测蛋白条带的免疫活性, 记录平均吸光度(Mean optical density)进行统计; 相对蛋白量=靶蛋白*D*值/相应β-actin蛋白*D*值。

1.7 统计学分析

所得数据用均数±标准差($\bar{x}$±S.E.M.); 所有数据经计算机SPSS 11.0软件采用independent samples *t* test或one-way ANOVA联合post hoc fisher protected least significant difference test进行统计学分析; $P<0.05$认为差异有统计学意义。

2 结果

2.1 2APB对缺血再灌注时体外培养星形胶质细胞活力的影响

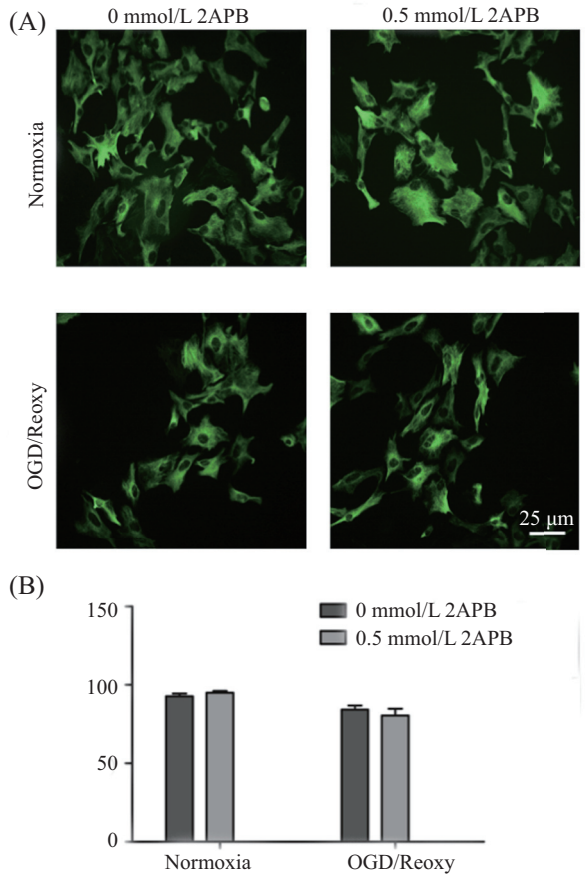
在OGD干预0, 0.5, 1, 2, 3, 6, 12 h进行观察, 可见随着OGD干预时间的延长, 星形胶质细胞胞体逐渐皱缩, 折光性降低, 细胞质内空泡增多; 培养皿中漂浮细胞和细胞碎片增多。OGD 2 h以内, OGD组星形胶质细胞形态与对照组无明显差异; 而OGD 6 h后, 视野中几乎见不到形态完整的星形胶质细胞。在OGD干预2 h以内, 2APB(0.5 mmol/L)+OGD组星形胶质细胞(图1A)与单纯OGD组相比, 略显狭长, 双极细胞增多; OGD 6 h后, OGD+2APB组星胶与单纯OGD组相似, 出现大量空泡, 细胞破碎甚至死亡。为排除细胞死亡对后续实验的影响, 我们选择OGD 2 h作为后续实验的干预时间。

进一步运用MTT检测OGD再灌注及2APB对体外培养星形胶质细胞活力的影响。如表1所示, OGD 2 h后再灌注0, 6, 12, 24, 48 h, 与Normoxia组相比, Normoxia+2APB组、OGD/Reoxy组 及OGD/Reoxy+2APB组细胞*D*值无显著性差异。说明: (1) OGD 2 h再灌注对体外培养星形胶质细胞活力无

表1 缺血再灌注(OGD/Reoxygenation)及2APB(0.5 mmol/L)处理不同时间后星形胶质细胞MTT *D*值的变化(mean±S.E.M., *n*=10)

Table 1 MTT *D* value of astrocytes treated with 2APB (0.5 mmol/L) under OGD/Reoxygenation (mean±S.E.M., *n*=10)

分组 Groups	时间 Times				
	0 h	6 h	12 h	24 h	48 h
Normoxia	0.136±0.009	0.143±0.005	0.144±0.011	0.145±0.016	0.155±0.020
Normoxia+2APB	0.148±0.037	0.138±0.005	0.162±0.057	0.159±0.020	0.163±0.040
OGD/Reoxygenation	0.145±0.022	0.136±0.008	0.156±0.022	0.152±0.012	0.186±0.026
OGD/Reoxygenation+2APB	0.131±0.012	0.141±0.028	0.151±0.011	0.160±0.013	0.185±0.029



A: OGD 2 h或正常培养情况下, 用0.5 mmol/L 2APB干预。GFAP免疫荧光染色观察体外培养星形胶质细胞活力的变化; B: OGD/Reoxy 48 h, 台盼蓝试验检测体外培养星形胶质细胞活力的变化。结果用mean±S.E.M.表示, *n*=5。

A: the cells were stimulated with 0.5 mmol/L 2APB for 2 h under normoxia or OGD. The morphology of the cells were analyzed by immunocytochemical staining of GFAP; B: viability of the cells were measured by trypan blue exclusion method. Mean±S.E.M., *n*=5.

图1 星形胶质细胞形态和活力的变化

Fig.1 Morphology and viability of astrocytes

明显影响; (2)0.5 mmol/L 2APB对正常培养及OGD/Reoxygenation情况下体外培养星形胶质细胞活力无明显影响。此外, 用台盼蓝染色鉴定细胞活力, 结果表明, 与MTT染色结果一致, OGD 2 h/Reoxy 48 h或OGD 2 h/Reoxy 48 h+2APB对体外培养星形胶质细胞活力无明显影响(图1B)。

2.2 2APB对脑缺血再灌注(OGD/Reoxy)时体外培养星形胶质细胞TRPV2表达的影响

在OGD/Reoxy 48 h后提蛋白, 检测2APB对OGD/Reoxy情况下TRPV2蛋白表达的影响。如图2所示, OGD/Reoxy 48 h后, Normoxia组、Normoxia+2APB组、OGD/Reoxy组 和OGD/Reoxy+2APB组TRPV2蛋白的表达没有明显差异, 提示2APB对脑缺

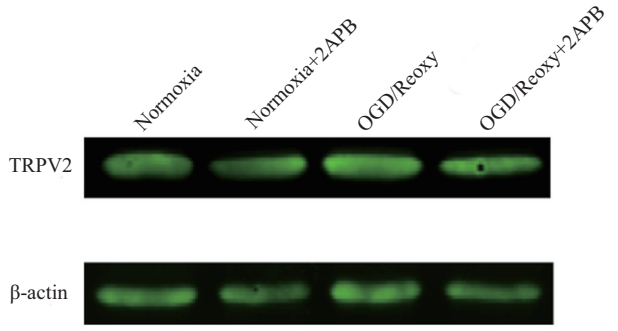


图2 缺血再灌注(OGD/Reoxygenation)及2APB干预48 h后星形胶质细胞TRPV2总蛋白表达的变化

Fig.2 The expression of TRPV2 treated with 2APB in primary cultured astrocytes under OGD/Reoxygenation

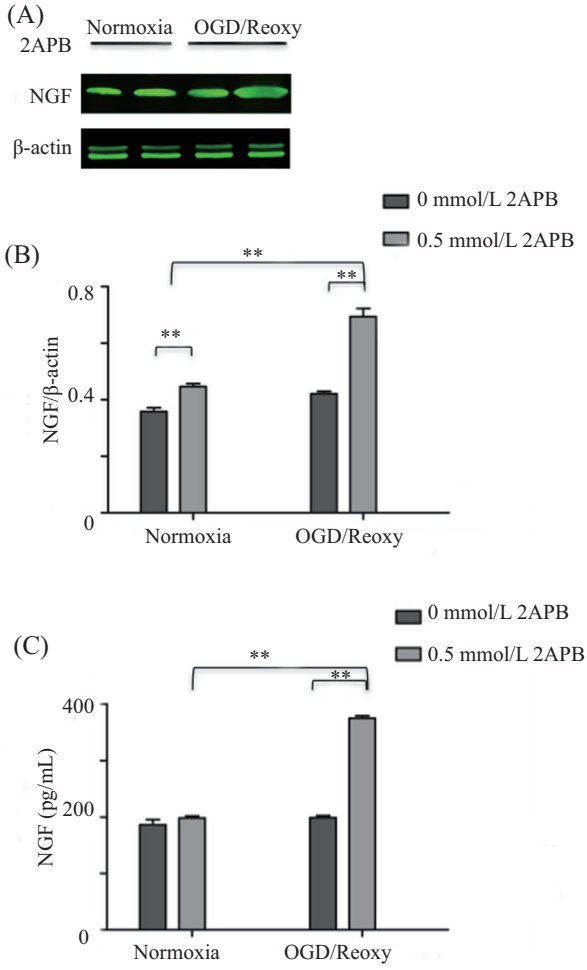
血再灌注时体外培养星形胶质细胞的表达量无明显影响。

2.3 2APB对脑缺血再灌注(OGD/Reoxygenation)时体外培养星形胶质细胞NGF合成和释放的影响

我们通过Western blot和ELISA实验对缺血再灌注(OGD/Reoxygenation)情况下体外培养星形胶质细胞NGF的合成与分泌进行了检测, 结果发现: 如图3A、3B所示, 0.5 mmol/L 2APB可以增加脑缺血再灌注情况下NGF蛋白的合成。虽然正常培养下, 2APB也可以增加星形胶质细胞NGF的合成(增加了0.088, $P<0.001$), 但这一趋势在缺氧再灌注的情况下更加明显(增加了0.273, $P<0.001$)。如图3C所示, 正常培养情况下, 2APB组星形胶质细胞条件性培养液中NGF蛋白的含量虽然有所增加, 但没有统计学差异(186±9.16 for control group *vs* 198±3.69 for 2APB group, $P>0.05$); 而缺血再灌注(OGD/Reoxygenation)的情况下, 2APB组星形胶质细胞条件性培养液中NGF的含量是未加2APB组的1.88倍($P<0.001$), 提示0.5 mmol/L 2APB可以增加缺血再灌注(OGD/Reoxygenation)情况下体外培养星形胶质细胞NGF的分泌。

2.4 MAPK信号途径阻滞剂对2APB诱导脑缺血再灌注(OGD/Reoxy)时NGF的释放的影响

既往研究表明, MAPK信号系统与星形胶质细胞NGF的合成和释放密切相关[11]。为进一步研究MAPK信号系统与2APB诱导脑缺血再灌注(OGD/Reoxy)时NGF释放的相互关系, 我们同时加入2APB和各种MAPK信号途径抑制剂, 并在缺血再灌注(OGD/Reoxy) 48 h后检测星形胶质细胞条件性培养液中NGF的含量。如图4所示, JNK抑制剂可以降低



A: 用0.5 mmol/L 2APB处理正常培养或缺血再灌注(OGD/Reoxy) 48 h后的星形胶质细胞, 通过Western blot分析星形胶质细胞NGF蛋白表达的变化; B: 相对NGF蛋白表达量=NGF吸光度/β-actin吸光度, 据此统计Western blot结果。结果用mean±S.E.M.表示, n=5。**P<0.01; C: 用0.5 mmol/L 2APB处理正常培养或缺血再灌注(OGD/Reoxygenation) 48 h后的星形胶质细胞, 通过ELISA检测星形胶质细胞条件性培养液中NGF的含量。结果用mean±S.E.M.表示, n=5。**P<0.01。

A: astrocytes, cultured under normoxia or hypoxia, were treated with 0.5 mmol/L 2APB for 2 h and then recovered to normal culture conditions with 2APB for 48 h. The NGF protein of astrocytes were analyzed by Western blot; B: quantitative analysis of NGF protein for Western blot. Values are expressed in terms of the ratio to the D value of β-actin. Mean±S.E.M., n=5. **P<0.01; C: the levels of NGF protein determined by ELISA. Mean±S.E.M., n=5. **P<0.01.

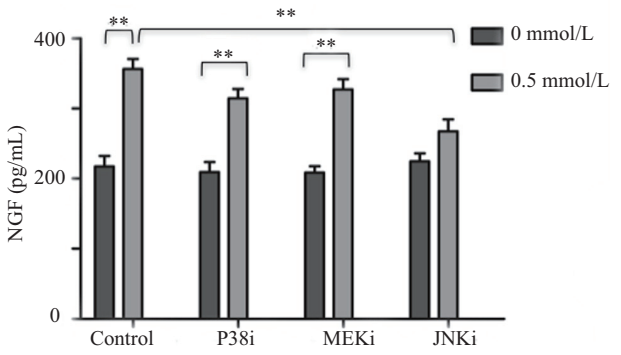
图3 缺血再灌注(OGD/Reoxygenation)及2APB干预48 h后体外培养星形胶质细胞NGF合成和释放的变化

Fig.3 The expression and release of NGF treated with 2APB in astrocytes under OGD/Reoxygenation

2APB诱导脑缺血再灌注(OGD/Reoxy)时NGF的释放。

2.4 2APB可增加JNK蛋白的磷酸化

如图5所示, 0.5 mmol/L 2APB可以增加脑缺血再灌注0.5 h后体外培养星形胶质细胞的pJNK蛋白

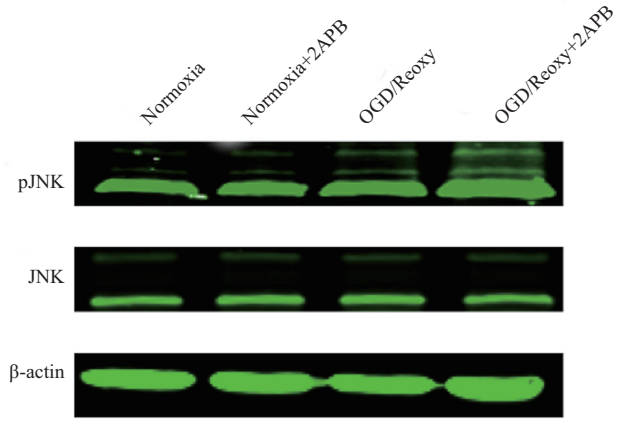


同时用0.5 mmol/L 2APB和10 μmol/L p38 MAPK抑制剂SB203580, 或10 μmol/L MEK1/2抑制剂U0126, 或10 μmol/L JNK抑制剂处理体外培养星形胶质细胞, 缺血再灌注(OGD/Reoxygenation) 48 h后, 通过ELISA检测星形胶质细胞条件性培养液中NGF的含量。实验结果用mean±S.E.M.表示, n=5。**P<0.01。

While subjected to OGD/Reoxygenation, the astrocytes were treated with p38 MAPK inhibitor SB203580 (10 μmol/L), MEK1/2 inhibitor U0126 (10 μmol/L) or JNK inhibitor (10 μmol/L) along with the treatment with 2APB (0.5 mmol/L). Cell-conditioned media were collected and measured for NGF protein by ELISA. Mean±S.E.M., n=5. **P<0.01.

图4 MAPK途径抑制剂对2APB诱导脑缺血再灌注(OGD/Reoxygenation)时NGF释放的影响

Fig.4 Effects of MAPK pathway inhibitors on the NGF expression in astrocytes treated with 2APB under OGD/Reoxygenation



用0.5 mmol/L 2APB处理体外培养星形胶质细胞, 缺血再灌注(OGD/Reoxygenation) 0.5 h后, 通过Western blot分析星形胶质细胞pJNK蛋白的表达。

The cells subjected OGD/Reoxygenation were treated with 0.5 mmol/L 2APB. After 0.5 h, cell extracts were analyzed for the levels of pJNK and total JNK proteins by Western blot. β-Actin was analyzed as a loading control.

图5 2APB对脑缺血再灌注时体外培养星形胶质细胞pJNK蛋白表达的影响

Fig.5 Effects of 2APB on the expression of phosphor-JNK (pJNK) protein in human astrocytes under OGD/Reoxygenation

表达水平, 这与JNK抑制剂可以降低2APB诱导脑缺血再灌注(OGD/Reoxygenation)时NGF的释放的结果相一致。

3 讨论

星形胶质细胞是中枢神经系统数量最多的细胞, 对维持大脑功能的稳定发挥着重要作用。星形胶质细胞对神经元起着重要的支持作用: 一方面, 星形胶质细胞通过将血液中的葡萄糖转化为乳酸向神经元提供营养供应; 另一方面, 星形胶质细胞还通过合成多种细胞营养因子对血管神经单元其他组分起到支持和保护的作用[12-15]。正因如此, 研究参与星形胶质细胞神经营养因子合成和释放的机制及其调控对维持大脑正常功能及促进中枢神经系统疾病后神经功能的恢复都有重要的意义。

现有研究表明, TRPV2在星形胶质细胞表面有丰富表达, 与星形胶质细胞钙活动相关[16-17]。但是, 关于星形胶质细胞TRPV2在脑缺血时的具体功能目前还不清楚。有研究发现, 缺氧刺激下, NGF可通过Ca^{2+}依赖性途径释放[18], 从而促进神经元的存活, 我们推测, 脑缺血再灌注时, 调控星形胶质细胞TRPV2活性可通过影响星形胶质细胞Ca^{2+}活动而影响其NGF的释放。

为验证上述假说, 我们研究了TRPV2激活剂2APB对脑缺血再灌注时体外培养星形胶质细胞NGF合成和释放的影响。我们首先检测了2APB对脑缺血再灌注时星形胶质细胞TRPV2蛋白表达的影响, 结果发现, 单纯OGD 2 h后再灌注及该条件下2APB的应用并不足以引起星形胶质细胞TRPV2蛋白表达的变化, 2APB对TRPV2的作用主要体现在对其活性的调控上[19]。进一步研究发现, TRPV2激活剂2APB可以诱导正常培养情况下及缺血再灌注情况下大鼠皮层星形胶质细胞NGF合成和释放的增加。既往研究表明, 病理情况下, NGF对神经元的存活起保护作用[2,20]。因此, 我们推测, 脑缺血再灌注时, 2APB可能通过激活星形胶质细胞表面表达的TRPV2, 对周围神经元及血管起到支持和保护作用。此外, 我们的研究还发现, 2APB对脑缺血再灌注情况下体外培养星形胶质细胞的形态无明显影响, 提示了低浓度2APB应用的安全性。

星形胶质细胞NGF表达的增加主要是通过MAPK信号途径[11]。研究表明, p38 MAPK途径和JNK途径与细胞增殖和衰老密切相关[21-23]。JNK途径可被许多细胞外刺激所激活, 一旦激活, JNK可通过磷酸化许多转录因子, 在转录水平调节基因的表达[24-25]。 在本研究中, JNK阻滞剂可以抑制2APB诱导脑缺血再灌注时星形胶质细胞NGF释放的增加, 同时, 2APB可以增加脑缺血再灌注情况下星形胶质细胞pJNK蛋白的表达, 提示脑缺血再灌注时, 激活TRPV2可能通过JNK依赖途径诱导体外培养星形胶质细胞NGF释放的增加, 从而对神经元和血管起到支持和保护作用。

综上所述, 我们认为, 脑缺血再灌注情况下, TRPV2激活剂2APB可以诱导体外培养星形胶质细胞NGF的合成和释放, 提示TRPV2活化在脑缺血再灌注情况下可能发挥脑保护作用。

参考文献 (References)

1 Villoslada P, Genain CP. Role of nerve growth factor and other trophic factors in brain inflammation. Prog Brain Res 2004; 146: 403-14.

2 Brown A, Ricci MJ, Weaver LC. NGF message and protein distribution in the injured rat spinal cord. Exp Neurol 2004; 188(1): 115-27.

3 Lykissas MG, Batistatou AK, Charalabopoulos KA, Beris AE. The role of neurotrophins in axonal growth, guidance, and regeneration. Curr Neurovasc Res 2007; 4(2): 143-51.

4 Lee TH, Kato H, Chen ST, Kogure K, Itoyama Y. Expression of nerve growth factor and trkA after transient focal cerebral ischemia in rats. Stroke 1998; 29(8): 1687-96.

5 Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D. A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 1999; 398(6726): 436-41.

6 Moran MM, McAlexander MA, Bíró T, Szallasi A. Transient receptor potential channels as therapeutic targets. Nat Rev Drug Discov 2011; 10(8): 601-20.

7 Rudolphi KA, Schubert P. Modulation of neuronal and glial cell function by adenosine and neuroprotection in vascular dementia. Behav Brain Res 1997; 83(1/2): 123-8.

8 Carman-Krzan M, Wise BC. Arachidonic acid lipoxygenation may mediate interleukin-1 stimulation of nerve growth factor secretion in astroglial cultures. J Neurosci Res 1993; 34(2): 225-32.

9 Kliot M, Smith GM, Siegal JD, Silver J. Astrocyte-polymer implants promote regeneration of dorsal root fibers into the adult mammalian spinal cord. Exp Neurol 1990; 109(1): 57-69.

10 Patterson MK Jr. Measurement of growth and viability of cells in culture. Methods Enzymol 1979; 58: 141-52.

11 Galve-Roperh I, Haro A, Díaz-Laviada I. Induction of nerve growth factor synthesis by sphingomyelinase and ceramide in primary astrocyte cultures. Brain Res Mol Brain Res 1997; 52(1): 90-7.

12 Stobart JL, Anderson CM. Multifunctional role of astrocytes as

gatekeepers of neuronal energy supply. Front Cell Neurosci 2013; 7: 38.

13 Albrecht PJ, Dahl JP, Stoltzfus OK, Levenson R, Levison SW. Ciliary neurotrophic factor activates spinal cord astrocytes, stimulating their production and release of fibroblast growth factor-2, to increase motor neuron survival. Exp Neurol 2002; 173(1): 46-62.

14 Albrecht PJ, Murtie JC, Ness JK, Redwine JM, Enterline JR, Armstrong RC, *et al*. Astrocytes produce CNTF during the remyelination phase of viral-induced spinal cord demyelination to stimulate FGF-2 production. Neurobiol Dis 2003; 13(2): 89-101.

15 Fischer R, Wajant H, Kontermann R, Pfizenmaier K, Maier O. Astrocyte-specific activation of TNFR2 promotes oligodendrocyte maturation by secretion of leukemia inhibitory factor. Glia 2014; 62(2): 272-83.

16 Nedungadi TP, Dutta M, Bathina CS, Caterina MJ, Cunningham JT. Expression and distribution of TRPV2 in rat brain. Exp Neurol 2012; 237(1): 223-37.

17 Shibasaki K, Ishizaki Y, Mandadi S. Astrocytes express functional TRPV2 ion channels. Biochem Biophys Res Commun 2013; 441(2): 327-32.

18 Deng Q. Efficacy evaluation of Erigeron breviscapus on neurological function recovery after minimally invasive procedures for removal of intracranial hematoma. Chinese Journal of Practical Nervous Diseases 2009; 12: 82.

19 Benfenati V, Amiry-Moghaddam M, Caprini M, Mylonakou MN, Rapisarda C, Ottersen OP, *et al*. Expression and functional characterization of transient receptor potential vanilloid-related channel 4 (TRPV4) in rat cortical astrocytes. Neuroscience 2007; 148(4): 876-92.

20 Cheng B, Mattson MP. NGF and bFGF protect rat hippocampal and human cortical neurons against hypoglycemic damage by stabilizing calcium homeostasis. Neuron 1991; 7(6): 1031-41.

21 Matsumoto T, Turesson I, Book M, Gerwins P, Claesson-Welsh L. p38 MAP kinase negatively regulates endothelial cell survival, proliferation, and differentiation in FGF-2-stimulated angiogenesis. J Cell Biol 2002; 156(1): 149-60.

22 Wada T, Penninger JM. Mitogen-activated protein kinases in apoptosis regulation. Oncogene 2004; 23(16): 2838-49.

23 Wada T, Stepniak E, Hui L, Leibbrandt A, Katada T, Nishina H, *et al*. Antagonistic control of cell fates by JNK and p38-MAPK signaling. Cell Death Differ 2008; 15(1): 89-93.

24 Nishina H, Wada T, Katada T. Physiological roles of SAPK/JNK signaling pathway. J Biochem 2004; 136(2): 123-6.

25 Yoshida H, Metoki N, Ishikawa A, Imaizumi T, Matsumiya T, Tanji K, *et al*. Edaravone improves the expression of nerve growth factor in human astrocytes subjected to hypoxia/reoxygenation. Neurosci Res 2010; 66(3): 284-9.