丙戊酸钠慢性作用及停药后对C6神经胶质瘤细胞GAT-3及GABA-T mRNA表达的影响

丙戊酸钠(valproic acid sodium, VPA)是一种常用的广谱抗癫痫药，可有效地提高大脑中主要的抑制性神经递质——γ-氨基丁酸(GABA)的水平[1]。丙戊酸钠需长期规则服用，以保持稳定有效的血药浓度而达到控制癫痫发作的目的。治疗期间如果随意停药或换药过快，轻则病人会出现停药症状，重则导致病情反复甚至加重[2][3]，即停药反跳。此停药反跳机制目前尚不清楚。GABA作用的程度和持续时间受到GABA转运体(GABA transporter, GAT)的调节[4]，GAT可介导高亲和性的、Na^+/Cl^-依赖性的GABA摄取进入神经末梢和胶质细胞突起，使突触间隙中GABA的浓度保持在一定的水平。目前已知的GAT主要包括GAT-1、GAT-2、GAT-3三种类型。其中GAT-3主要分布在星形胶质细胞的突起上[5]，在GABA的摄取中起主要作用。被摄取的GABA可在GABA转氨酶(GABA transaminase, GABA-T)的作用下转化为琥珀酸半醛，后者再氧化为琥珀酸而进入三羧酸循环[6]。C6神经胶质瘤细胞常用于研究星形胶质细胞的功能和特性[7]。因此本实验通过研究VPA慢性作用及停药后对C6神经胶质瘤细胞GAT-3及GABA-T mRNA表达水平的影响，来探讨丙戊酸钠的停药反应机制。

1 材料与方法

1.1 细胞培养

C6神经胶质瘤细胞购买于ATCC公司，于37 ℃、5% CO_2条件下培养于含10%胎牛血清的DMEM培养基。

1.2 VPA慢性作用

VPA(Sigma)的有效血浆浓度为50~100 mg/L，本实验采用含50 mg/L VPA的DMEM培养基将C6细胞培养2周后制成VPA慢性作用模型[8]。然后将正常C6细胞及VPA慢性作用的C6细胞按2×105的密度接种于35 mm培养皿。培养48 h后，各停药组(分为停药30 min、6 h、12 h、24 h、48 h组)按相应时间停药，即用不含VPA的DMEM培养基漂洗3遍，再换以DMEM培养基继续培养。各组在接种后的细胞培养时间均为96 h。

1.3 RT-PCR

用Trizol(Gibical)法提取各组细胞总RNA，方法参照Trizol试剂说明书。提取的RNA用逆转录酶AMV(Promga)、 Oligo dT(18 mer)等合成cDNA第1链，反应条件：42 ℃ 1h，0 ℃ 5 min。GAT-3上游引物为5'-atg tgt gga gtt cca gaa gc-3'，下游引物为5'-cac acc tgt gga tca gag ag-3'，扩增片段为360 bp。PCR参数为：95 ℃ 30 s，55 ℃ 1 min，72 ℃ 1 min，30个循环。上游引物为5'-aac tac gaa gag agc cga gg-3'，下游引物为5'-gag agg atg ctg tag tct gg-3'，PCR产物为410 bp。其PCR参数为：95 ℃ 30 s，57 ℃ 1 min，72 ℃ 1 min，30个循环。内参为β-actin，其上游引物为5'-agc aag aga ggc atc ctg ac-3'，下游引物为5'-gtg gta cga cca gag gca ta-3'，扩增产物为268 bp。同样条件下用无Rnase水代替AMV作为阴性对照。实验重复3次。

1.4 结果分析

PCR扩增产物进行琼脂糖凝胶电脉并用BIO-PROFIL/BIO-CAPT/BIO-1D++图像分析软件(Vilber Lourmat 公司)对图像条带进行扫描。根据各组的GAT-3、GABA-T的相对灰度值[RV值，即GAT- 3、GABA-T电泳条带与相应的β-actin条带的灰度积分的百分比值，RV=(VGAT-3/Vβ-actin)×100%，以均数±标准差表示]，用Origin7.0软件作图，比较各组GAT-3和GABA-T mRNA表达水平的变化。

2 结果

2.1 对GAT-3 mRNA表达的影响

在VPA慢性作用下，RV值为(39.1±0.5)%，低于正常对照组的(46±1.3)%；各停药组的RV值均低于VPA慢性作用组，其中停药24 h组的最低，为(11.7±1.6)%，停药30 min组的最高，为(38.9±0.6)%，略低于VPA慢性作用组；而停药48 h组又升

高到(33.5±1.1)%，明显高于停药24 h组(图1)。

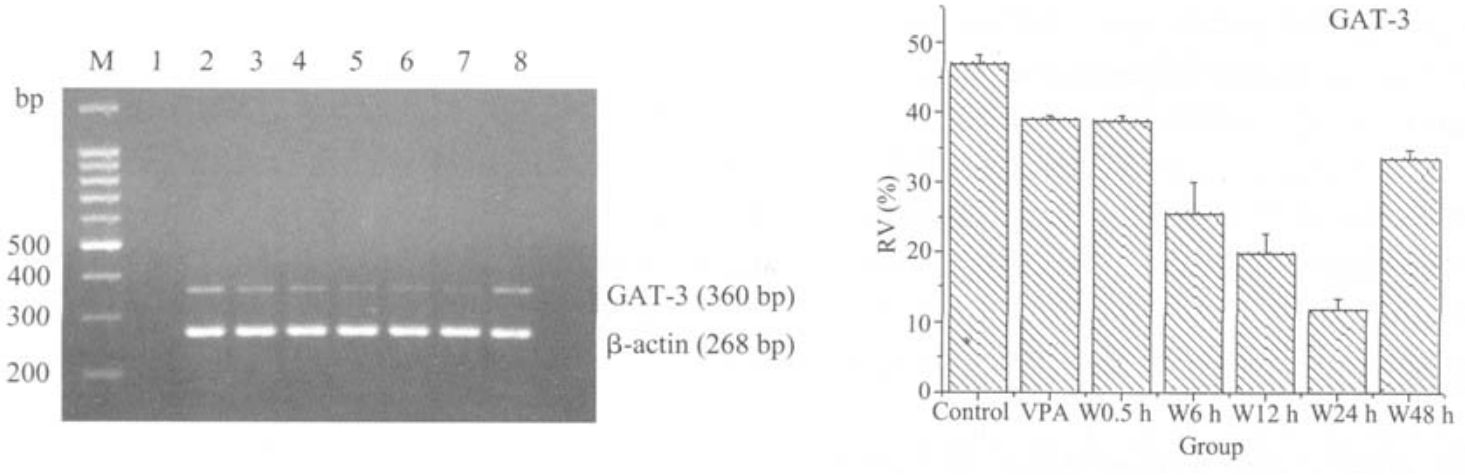

图1　VPA慢性作用及停药后对C6细胞GAT-3 mRNA表达的影响

Fig.1　Effects of chronic VPA treatment and withdrawal on GAT-3 mRNA expression in C6 glioma cells

Lane 1: Negative control; Lane 2: VPA-free C6 glioma (Control); Lane 3: Chronic treatment with VPA (VPA); Lane 4: VPA withdrawal 30 min (W-30 min); Lane 5: VPA withdrawal 6 h (W-6 h); Lane 6: VPA withdrawal 12 h (W-12 h); Lane 7: VPA withdrawal 24 h (W-24 h); Lane 8: VPA withdrawal 48 h (W-48 h)

2.2　对GABA-T mRNA表达的影响

VPA的慢性作用使GABA-T mRNA表达上调，对照组与VPA组的RV值分别为(34.77±2.36)%和(71.31±8.39)%。与VPA组相比，各停药组GABA-T mRNA表达水平明显下降；除停药12 h组[RV值(25.36±7.68)%]低于对照组外，其余停药组GABA-T mRNA表达水平均高于对照组(图2)。

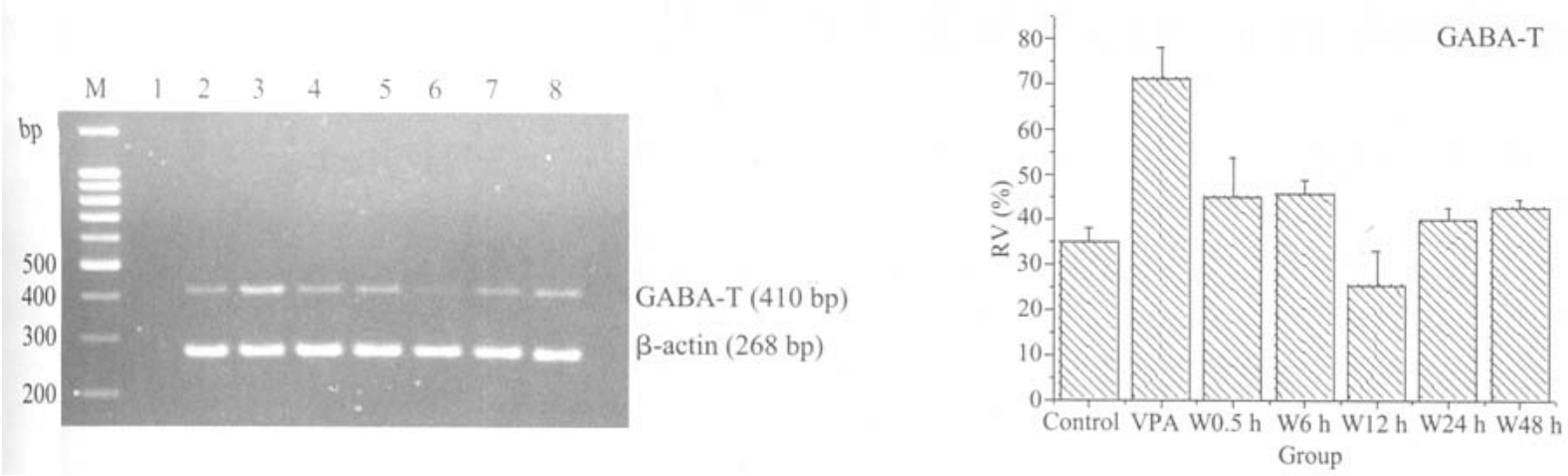

图2　VPA慢性作用及停药后对C6细胞GABA-TmRNA表达的影响

Fig.2　Effects of chronic VPA treatment and withdrawal on GABA-T mRNA expression in C6 glioma cells

Lane 1: Negative control; Lane 2: VPA-free C6 glioma (Control); Lane 3: Chronic treatment with VPA (VPA); Lane 4: VPA withdrawal 30 min (W-30 min); Lane 5: VPA withdrawal 6 h (W-6 h); Lane 6: VPA withdrawal 12 h (W-12 h); Lane 7: VPA withdrawal 24 h(W-24 h); Lane 8: VPA withdrawal 48 h (W-48)

3　讨论

GABA是中枢神经系统中主要的抑制性神经递质，估计有60%~75%的突触中存有GABA，而GABA能神经元功能的损伤造成脑内GABA水平低下与癫痫发生密切相关[9][10][11]。长期以来神经科学领域的研究，包括癫痫相关的病理学和神经药理学研究，一直集中于神经元上，而未对神经胶质细胞加以足够的重视。传统观念认为，神经胶质细胞是仅仅对神经元具有支持、营养和保护作用的非兴奋性细胞。1998年，Araque等[12]首次发现星形胶质细胞可通过释放谷氨酸等化学递质来调节神经元的活动。

现已证明，星形胶质细胞具有多种电压及配体依赖的离子通道，含有大部分神经递质、神经肽、激素及神经营养因子受体，并能分泌多种神经活性物质。因此，神经胶质细胞尤其是星形胶质细胞的作用日益受到关注。更考虑到星形胶质细胞在调节突触间隙GABA水平上重要的地位，所以本实验选择C6细胞，从星形胶质细胞的角度来探讨VPA停药反跳机制。本实验结果表明，VPA的慢性作用可抑制GAT-3 mRNA的表达。停药后GAT-3 mRNA水平继续下降，至停药48 h又开始明显上升。提示VPA通过GAT-3调节脑内GABA水平可能是其抗癫痫的作用机制之一。停药后GAT-3 mRNA表达的变化可能与停药反跳有关。GAT-3一方面可摄取突触间隙中的GABA，另一方面又可在一定条件如癫痫状态下逆向运转，释放GABA来调节突触的活动。因此，GAT-3 在停药反应中的具体作用仍需进一步研究。并为今后将GAT-3 作为一个重要的靶点来开发新的抗癫痫药物提供实验依据。本实验还观察到VPA的慢性作用使GABA-T mRNA表达上调，促进细胞内的GABA转变为琥珀酸，进入三羧酸循环后，使草酰乙酸生成增加，这可能会促进草酰乙酸在转氨酶的作用下生成天门冬氨酸[6]。星形胶质细胞可释放天门冬氨酸，并可通过NMDA受体激活抑制性中间神经元来抑制神经元过度兴奋，这也许是VPA另一抗癫痫作用途径。停药后GABA-T mRNA表达的变化也可能与停药反跳有关，但仍需深入研究。

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回结果列表