异丙肾上腺素在C2C12细胞分化中的作用及机制

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摘要 该研究主要探讨异丙肾上腺素(isoprenaline, ISO)在C2C12细胞分化与肌萎缩中的作用及可能的机制。在利用免疫组化分析C2C12细胞肾上腺素能受体表达特征的基础上, 以2%的马血清高糖培养基建立C2C12细胞分化的实验体系, 随后分别按单次或连续单次给予10^{-5} mol/L ISO后观察其分化差异; 接着再比较连续单次给予不同浓度的ISO(10^{-8} mol/L、10^{-7} mol/L、10^{-6} mol/L、10^{-5} mol/L)处理; 利用细胞免疫荧光化学和Western blot方法检测C2C12细胞分化后肌球蛋白重链(myosin heavy chain, MYH)的水平, 并定量分析分化后骨骼肌细胞的肌管细胞核融合数目; 同时, 利用Western blot检测C2C12细胞分化调节有关的肌细胞生成蛋白(myogenin, MyoG)、p-p38MAPK(phosphorylated p38-mitogen-activated protein kinase)及p-AKT(phosphorylated/protein kinase B)的水平变化。结果显示, C2C12细胞呈现出α-肾上腺素和β-肾上腺素能受体表达的特征。分化培养诱导下, C2C12细胞随着时间的延长, 分化成肌管的数量逐渐增多, 在第8 d达峰值。单次一次给予10^{-5} mol/L ISO没有连续单次给予ISO抑制C2C12细胞分化成骨骼肌细胞显著, 且连续单次给予ISO随着其浓度的增加, C2C12细胞分化为肌管细胞核融合数目逐渐减少并在ISO浓度为10^{-5} mol/L时最显著。与此同时, 随着连续单次给予不同浓度(10^{-8} mol/L、10^{-7} mol/L、10^{-6} mol /L、10^{-5} mol/L)的ISO, MYH和MyoG的表达水平呈现随着浓度增加而逐渐降低的特征, 而且p-p38MAPK及p-AKT的水平也呈连续单次给予ISO浓度增加而降低的趋势, 在ISO浓度为10^{-5} mol/L时降低最显著。该研究提示, 连续单次给予ISO显著抑制C2C12细胞分化为成熟骨骼肌细胞, 且与p-p38MAPK、p-AKT及MyoG水平的降低密切相关, 从而为交感神经长期过度兴奋所伴随的肌萎缩提供新的研究模型和治疗靶点。

关键词 异丙肾上腺素; 交感神经过度兴奋; 骨骼肌萎缩; C2C12细胞; 分化

Isoprenaline Induced Muscle Atrophy by Inhibiting the Differentiation of C2C12 Cells into Skeletal Muscle Cells

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Abstract This study is to investigate the effect and possible mechanism of isoprenaline (ISO) on C2C12 cells differentiation into skeletal muscle cells and muscle atrophy. Adrenergic receptors expressions in C2C12 cells were detected using immunofluorescence cytochemistry staining, and the cultured C2C12 cells that reached 70%

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confluence *in vitro* were used to establish the model of skeletal muscle stem/progenitor cells differentiation under 2% horse serum with high glucose DMEM. And then, using the cells differentiation model to compare the difference of either single or continuous single aderministration, single-dose (only one time) or continuous single-dose (one time each day for 8 days) of ISO with 10^{-5} mol/L were added into the differentiation medium. Subsequently, the different dosages effects of ISO with 10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L or 10^{-5} mol/L on the cells differentiation were performed by using aderministration of continuous single-dose. After that, immunofluorescence cytochemistry staining were used to analyze the expression of myosin heavy chain (MYH) in differentiated cells from C2C12 cells, and the quantitative analysis of the number of nucleus of myotubes of skeletal muscle cells. Western blot was used to detect the expression of p-p38MAPK (phosphorylated p38-mitogen-activated protein kinase), p-AKT (phosphorylated protein kinase B), MYH and myogenin (MyoG). C2C12 cells showed the traits of α1-, α2-, β1- and β2-subtypes of adrenergic receptors expressions. C2C12 cells gradually differentiated into mature skeletal muscle cells during the procedure of differentiation condition, reaching the peak at day 8 of differentiation period. Single-dose ISO with 10^{-5} mol/L slightly inhibited C2C12 cells differentiations into myotubes compared to continuous single-dose ISO. Furthermore, C2C12 cells differentiations into myotubes were gradually inhibited when exposed to continuous single-dose ISO with 10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L, 10^{-5} mol/L, showing dose-dependent manner. Furthermore, the numbers of myotubes with the different nuclear fusion numbers were gradually reduced when the differentiating C2C12 cells were exposed to the stimulation of different dosages of continuous single-dose ISO. Especially at the 10^{-5} mol/L continuous single-dose ISO, the potential of differentiation of C2C12 cells into mature skeletal muscle cells were markedly weaken. Simultaneously, the levels of p-p38MAPK and p-AKT, and the expressions of MYH and MyoG as determined by Western blot were dosages-dependently decreased in the differentiating C2C12 cells with aderministration of continuous single-dose ISO. It is suggested that ISO, especially with continuous aderministration, inhibited C2C12 cells differentiation into mature skeletal muscle cells, which was associated with the reduced p-p38MAPK and p-AKT levels, and decreased MyoG expressions. These results provided a new therapeutic target for prolonged sympathetic overactivity-related muscle atrophy.

Keywords isoprinosine; sympathetic overactivity; skeletal muscle atrophy; C2C12 cells; differentiation

肌营养不良是一种破坏性的神经肌肉疾病, 其特征为肌肉收缩无力和肌肉萎缩的进行性加重。在许多肌营养不良患者中已观察到心肌疾病的发生, 主要表现为扩张型心肌病(dilated cardiomyopathy, DCM)、心力衰竭(心衰)或传导系统异常等[1-2]。心衰是多种心血管疾病的严重和终末阶段, 其临床特征为呼吸困难、体力活动受限、易疲劳和骨骼肌萎缩等[3], 但心衰与骨骼肌萎缩的关系至今仍不十分清晰。

从基础到临床的研究发现, 心衰常涉及心肌损伤和心输出量降低, 其结果导致交感神经代偿性增加神经活动, 即自主神经功能障碍(交感神经兴奋增加或副交感神经兴奋减少)[4-5], 这一失衡又进一步恶化心脏的结构和功能。更重要的发现是, 短期的交感神经活性增加有利于改善骨骼肌的质量。相反, 长期慢性交感神经活性增加对机体却是有害的, 例如在心衰时表现为肌肉萎缩[6]。然而遗憾的是, 至今没有建立一个模拟在体长期交感神经活性增加的体外细胞模型。

实际上, 心衰时增强的交感神经活性降低了骨骼肌β2肾上腺素能受体(beta 2 adrenergic receptors, β2-AdR)的表达和敏感性, 减少了β2-AdR所介导骨骼肌的合成代谢[7]。更有证据显示, 交感神经过度兴奋导致心衰相关的骨骼肌病, 其原因可能是导致心衰患者慢性血管收缩[8]、与骨骼肌氧化应激相关[9]以及促炎细胞因子浓度增加[10]。但是, 有关交感神经长期过度兴奋时骨骼肌干细胞分化能力有无变化未见报道。本课题利用异丙肾上腺素(isoprenaline, ISO)模拟交感神经长期过度兴奋的状态, 在此状态下研究C2C12细胞分化功能的变化特征与肌萎缩的关系, 期望以此为交感神经长期过度兴奋造成血液中过多的去甲肾上腺素和肾上腺素参与骨骼肌萎缩的过程提供新的实验依据, 从而为交感神经长期过

度兴奋(即自主神经功能障碍)时所伴随的肌萎缩提供新的理论依据。

1 材料与方法

1.1 材料

C2C12成肌细胞系(简称C2C12细胞)购自中国科学院上海细胞库; 胎牛血清购自杭州四季青生物工程材料有限公司; 马血清购自美国Gibco公司; 0.25%胰蛋白酶购自武汉安特捷生物技术有限公司; 荧光倒置显微镜购自日本尼康公司; 高糖DMEM购自美国Gibco公司。

1.2 方法

1.2.1 C2C12细胞的培养与分化体系的建立

C2C12细胞常规复苏并接种于75 cm^2的培养皿中, 培养条件为10%胎牛血清+高糖DMEM培养基, 置于37 ℃、5% CO_2的饱和湿度细胞培养箱中培养。待细胞增殖融合达90%~100%时, 按1:3传代。传代的C2C12细胞按上述条件继续培养, 以备后续实验用。

C2C12细胞在10%胎牛血清高糖DMEM培养基中培养, 待细胞生长至70%融合时, 换用2%的马血清诱导C2C12细胞分化。每隔1 d换液1次, 同时在显微镜下观察细胞的分化情况, 并分别于第3、5、8 d时收取蛋白, 免疫印迹法检测C2C12细胞分化指标的情况。

1.2.2 给药方式及分组 在增殖培养基中C2C12细胞增殖融合至70%时, 换成2%马血清高糖DMEM培养, 分别加入异丙肾上腺素, 按单次给药和连续单次给药诱导5~8 d后, 按检测指标进行处理分析。给药模式分为两种, 单次给药和连续单次给药。单次给药: 在换成2%马血清高糖DMEM时加入异丙肾上腺素, 随后每隔1 d换成2%马血清高糖DMEM, 不再加异丙肾上腺素; 连续单次给药: 在换成2%马血清高糖DMEM时加入异丙肾上腺素, 随后每隔1 d换成2%马血清高糖DMEM, 同时再加异丙肾上腺素。

C2C12细胞传代后, 在增殖培养基中培养至70%, 用2%马血清加不同浓度的异丙肾上腺素分成5组[对照组(ISO 0 mol/L)、ISO 10^{-8} mol/L组、ISO 10^{-7} mol/L组、ISO 10^{-6} mol/L组、ISO 10^{-5} mol/L组], 每隔1 d换液1次, 并同时加入相应浓度的异丙肾上腺素诱导8 d后, 按检测指标进行处理分析。

1.2.3 细胞免疫荧光化学检测 C2C12细胞按每孔1.25×10^4/mL密度接种于24孔板中, 待细胞贴壁完全铺开24 h后, 4%的多聚甲醛固定15 min, 然后1×PBS洗涤3次, 每次5 min, 再用5%的山羊血清和0.3% Triton X-100封闭30 min。封闭后的细胞每孔分别加入抗体稀释液稀释的肌球蛋白重链(myosin heavy chain, MYH)(货号: sc-20641, 抗体稀释1:50, Santa Cruze公司)、α1-肾上腺素能受体(alpha 1 adrenergic receptors, α1-AdR)(货号: ab137123, 抗体稀释1:100, Abcam公司)、α2-AdR(货号: ab56871, 抗体稀释1:100, Abcam公司)、β1-AdR(货号: ab3442, 抗体稀释1:100, Abcam公司)、β2-AdR(货号: ab36956, 抗体稀释1:100, Abcam公司)、β3-AdR(货号: ab59685, 抗体稀释1:100, Abcam公司)等抗体, 4 ℃过夜。第2 d, 1×PBS洗涤3次, 每次5 min, 再加入抗兔的荧光二抗(1:150), 室温孵育2 h, 1×PBS洗涤3次, 每次5 min, 最后加入150 μL 50 μg/mL的DAPI , 避光室温30 min后, 换成1×PBS在倒置荧光显微镜下观察并摄片。另一组C2C12细胞用24孔板接种细胞后, 在增殖培养基中增殖融合至70%后, 换成2%马血清高糖DMEM培养, 分别加入不同浓度的异丙肾上腺素(对照分化组、ISO 10^{-8} mol/L组、ISO 10^{-7} mol/L组、ISO 10^{-6} mol/L组、ISO 10^{-5} mol/L组), 每隔1 d换1次液, 诱导5~8 d后, 用同样的方法在荧光显微镜下观察各组的分化情况。并利用Image Pro图像分析软件半定量分析融合肌管细胞核的数目。

1.2.4 Western blot分析 C2C12细胞经处理后, RIPA裂解后离心收蛋白, 并用BCA试剂盒进行蛋白定量。每泳道上样50 μg蛋白。然后经10% SDS-PAGE凝胶电泳后, 转移到PVDF膜上。用TBST配制的5%的脱脂奶粉低速摇床封闭40 min, 将封闭好的PVDF膜分别放入含有α-Tubulin(货号: T6074, 抗体稀释1:5 000, Sigma公司)、AKT(protein kinase B)(货号: #9272s, 抗体稀释1:500, Cell Signaling Technology公司)、p-AKT(phosphorylated protein kinase B)(货号: #9271s, Ser473, 抗体稀释1:500, Cell Signaling Technology公司)、p38MAPK(p38-mitogen-activated protein kinase)(货号: #8690, 抗体稀释1:500, Cell Signaling Technology公司)、p-p38MAPK(phosphorylated p38-mitogen-activated protein kinase)(货号: #9211, 抗体稀释1:500, Cell Signaling Technology公司)、MYH(myosin heavy chain)(货号: sc-20641, 抗体稀释1:500, Santa Cruze公司)和MyoG(myogenin)(货号: sc-12732, 抗体稀释1:500, Santa Cruze公司)等不同一抗的封闭袋中, 4 ℃过夜。然后用TBST于脱色摇床中洗3次, 每次10 min, 再将

条带放入含有辣根过氧化酶标记的二抗(1:10 000)孵育液中2 h, 用TBST洗3次, 每次10 min, 最后用TBST在摇床中洗3次, 每次10 min。根据辣根过氧化酶显色剂的化学发光法在Bio-Rad蛋白成像仪成像, 并用Image J软件测灰度值进行半定量分析。

1.3 统计学方法

采用SPSS 17.0软件进行统计学分析, 计量资料以均数±标准差表示, 多组间比较采用单因素方差分析(One-Way ANOVA), 两组间比较采用*t*检验。$P<0.05$表示差异有统计学意义。

2 结果

2.1 C2C12细胞的培养与分化体系的建立

C2C12细胞经常规传代后接种在75 cm^2的培养皿中, 在倒置显微镜下观察发现, 细胞贴壁铺开后多呈梭形或有突起, 核为椭圆形, 单核, 核仁明显。待细胞融合至70%时, 更换2%马血清高糖DMEM诱导分化液。分化的细胞呈现多核、串珠样排列, 并形成肌管。加入分化液后约第3 d, C2C12细胞成队列排齐, 开始准备分化, 第4 d可在光镜下见到肌管形成, 随着时间的推移肌管细胞核融合数目逐渐增多, 分化第8 d达到高峰(图1)。收取C2C12细胞增殖、分化第3、5、8 d的蛋白, Western blot检测结果显示, 处于细胞增殖期的MYH的表达量相对少, 而C2C12细胞处于细胞分化时随着时间的增加肌球蛋白重链(myosin heavy chain, MYH)逐渐增加, 并在第8 d达到顶峰(图2)。

2.2 肾上腺素能受体在C2C12细胞的表达特征

细胞免疫荧光染色显示, C2C12细胞呈现出β1-AdR、β2-AdR、α1-AdR、α2-AdR阳性的特征(图3)。

2.3 ISO对C2C12细胞分化的影响

细胞免疫荧光结果显示, 在分化培养基作用第8 d时, 明显观察到C2C12细胞分化为成熟的骨骼肌细胞, 呈现出多核、成串珠样排列的肌管(图4)。在给予异丙肾上腺素(ISO)干预后发现, 随着ISO浓度的增加, C2C12细胞分化为成熟骨骼肌细胞的能力均有所抑制, 但连续单次给予ISO明显强于单次给予ISO(图4)。连续单次给ISO明显抑制肌管形成, 并呈现出肌管细胞核融合数目小于5的肌管逐渐增多而肌管细胞核融合数目大于10的肌管逐渐减少的趋势, 且ISO浓度为10^{-5} mol/L时效果最明显(图5)。

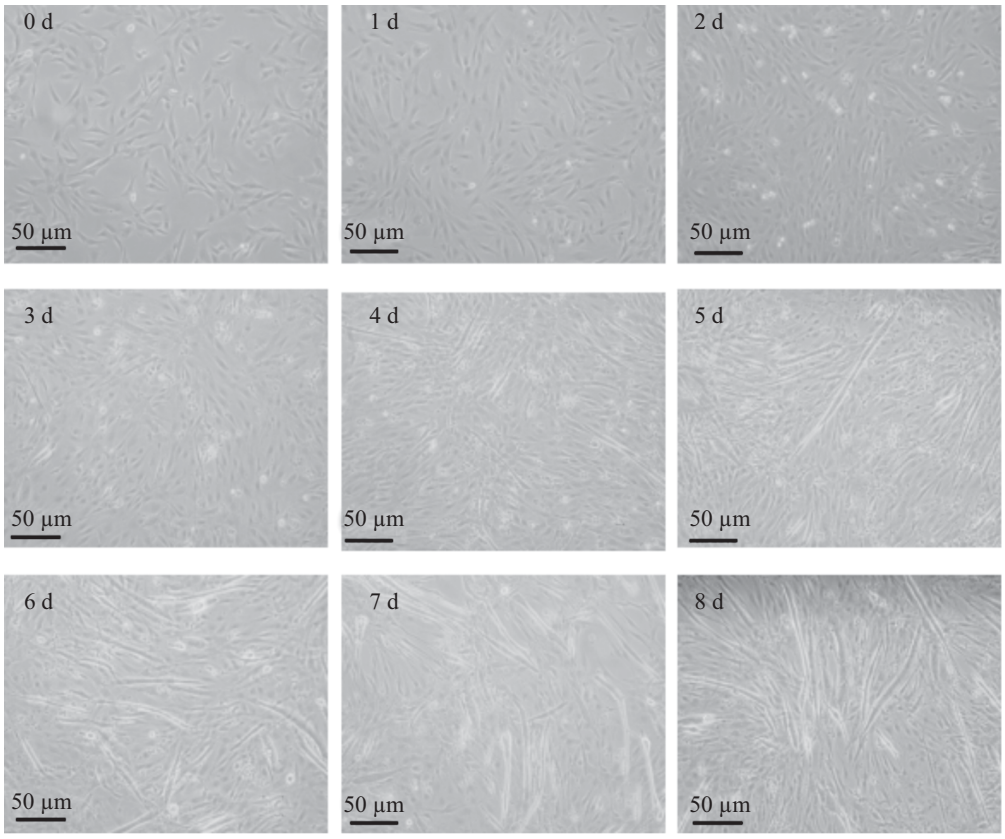
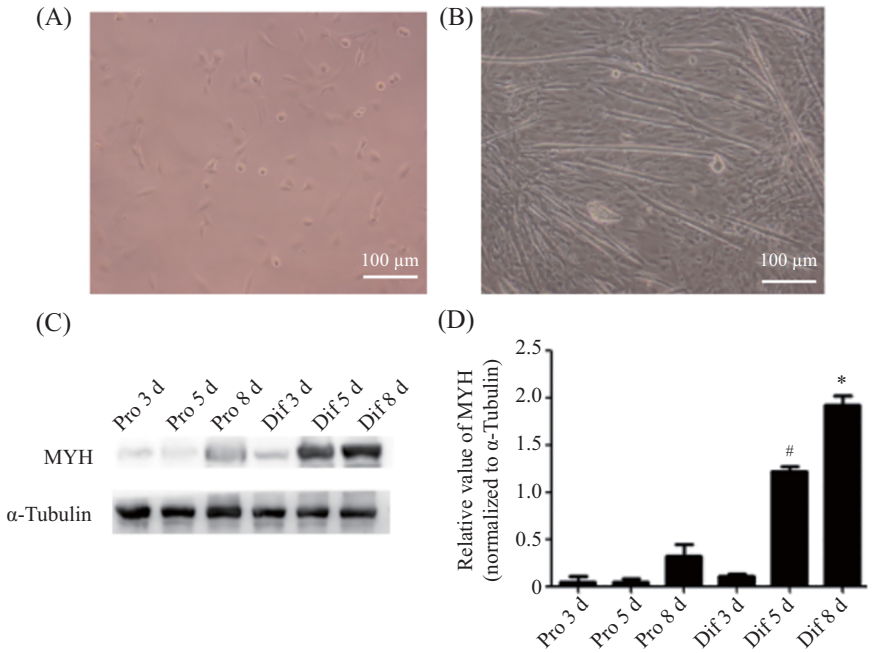


图1 光镜下C2C12细胞分化的时间窗

Fig.1 Time window of C2C12 cells differentiation under light microscope



A: 增殖状态下的C2C12细胞。B: 分化状态下的C2C12细胞。C: Western blot检测分析增殖及分化条件下C2C12细胞MYH的水平。D: 半定量分析显示MYH的水平; $^{\#}P<0.05$, 与分化3 d组比较; $^{*}P<0.05$, 与分化5 d组比较。Pro: 增殖; Dif: 分化。

A: typical image for proliferating C2C12 cells. B: typical image for differentiating C2C12 cells. C: the levels of MYH were detected under proliferation and differentiation condition of C2C12 cells by Western blot. D: semi-quantitative analysis in MYH levels; $^{\#}P<0.05$ *vs* dif 3 d group; $^{*}P<0.05$ *vs* dif 5 d group. Pro: proliferation; Dif: differentiation.

图2　C2C12细胞的培养与分化体系的建立

Fig.2　Cells culture and establishment of C2C12 cells differentiation system

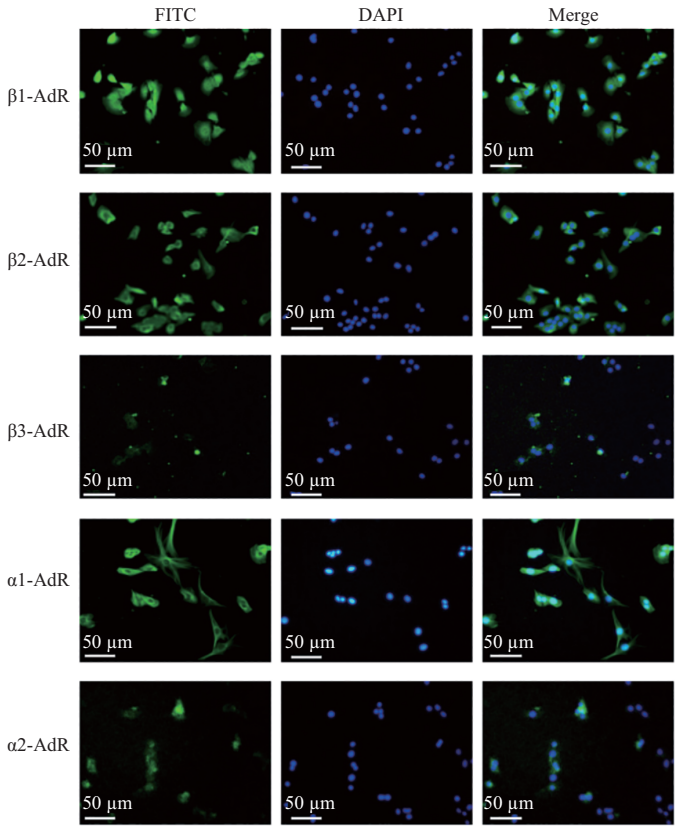
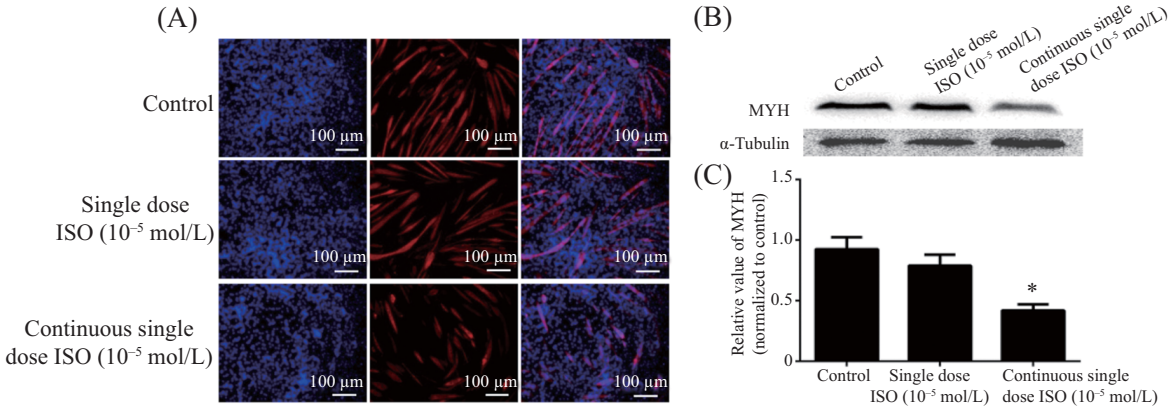


图3　C2C12细胞肾上腺素能受体表达特征

Fig.3　Expression traits adrenergic receptors in C2C12 cells

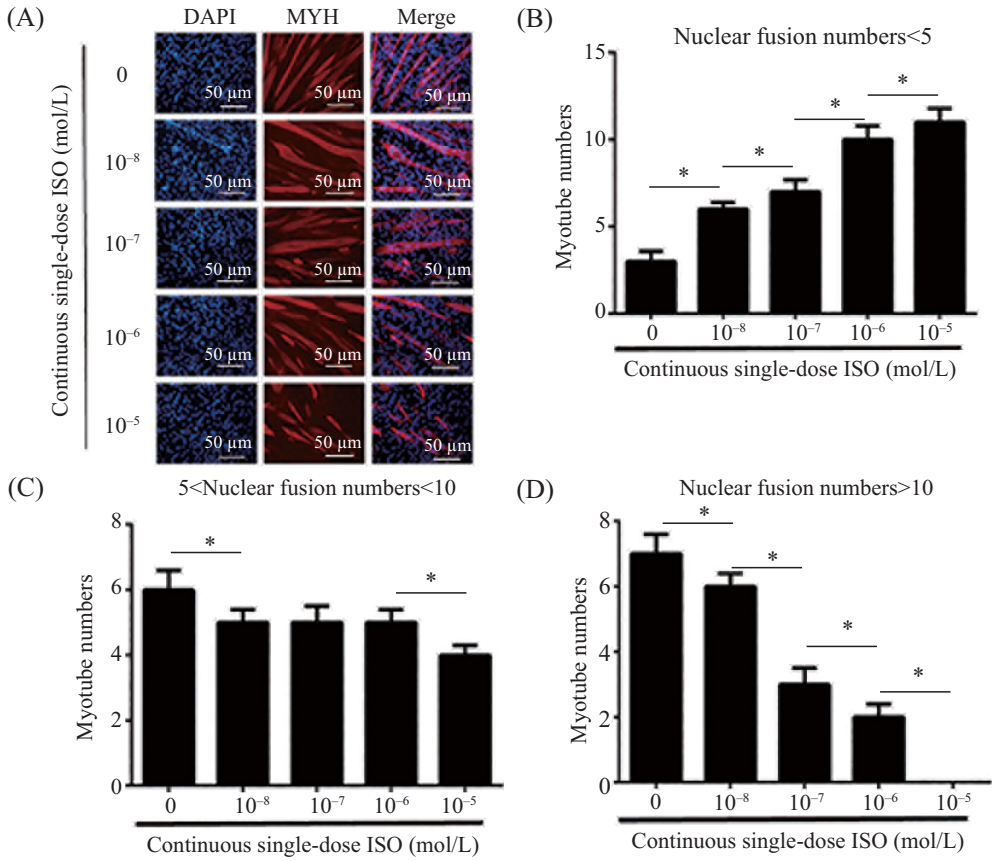


A: 单次或连续单次给予ISO抑制C2C12细胞分化的典型免疫荧光细胞化学染色图片; B: Western blot检测分析单次或连续单次给予ISO条件下C2C12细胞MYH的水平变化; C: 半定量分析显示MYH水平变化特征, n=3, *P<0.05, 与单次给ISO 10^{-5} mol/L组比较。

A: typical pictures of C2C12 cells differentiation into mature skeletal muscle cells by immunofluorescence cytochemical staining; B: the levels of MYH in C2C12 cells were detected in response to single-dose or continuous single-dose ISO by Western blot; C: semi-quantitative analysis showed traits of MYH levels in different aderministration of ISO, n=3, *P<0.05 *vs* single-dose 10^{-5} mol/L group.

图4 单次或连续单次给予ISO对C2C12细胞分化的影响

Fig.4 The effect of single-dose or continuous single-dose ISO on C2C12 cells differentiation

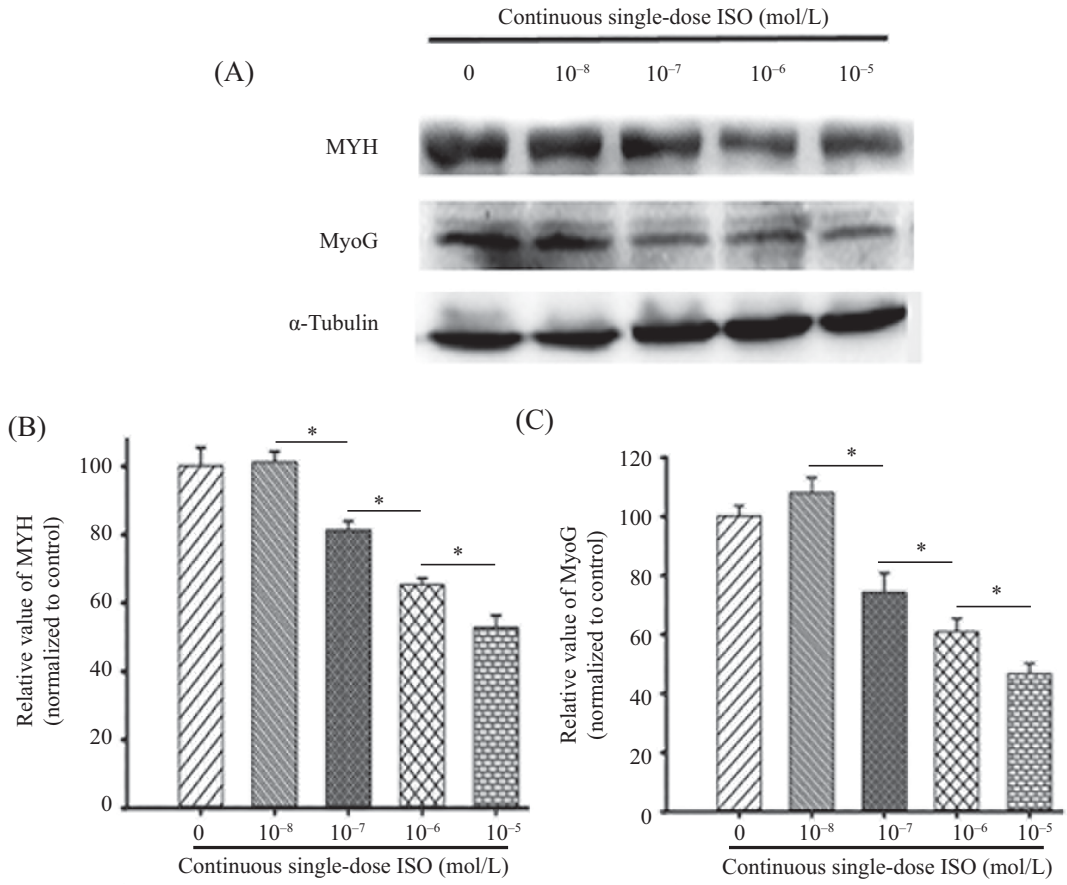


A: 连续单次给不同剂量(10^{-8} mol/L、10^{-7} mol/L、10^{-6} mol/L 、10^{-5} mol/L)ISO逐渐抑制C2C12细胞分化的MYH免疫荧光细胞化学染色, 红色荧光为MYH阳性, 蓝色荧光为DAPI染的细胞核; B~D: 连续单次给不同剂量ISO对C2C12细胞分化为含有不同数目细胞核融合的肌管数的影响, n=6, *P<0.05。

A: immunofluorescence cytochemical staining of MYH in the gradually inhibitory differentiation of C2C12 cells exposed to the stimulation of continuous single-dose (10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L, 10^{-5} mol/L) ISO, red fluorescence indicated MYH positive differentiated C2C12 cells, blue fluorescence indicated DAPI- labeled nucleus; B-D: the effect of continuous single-dose ISO (10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L, 10^{-5} mol/L) on the numbers of myotube with the indicated nuclear fusion numbers during C2C12 cells differentiation, n=6, *P<0.05.

图5 连续单次给予ISO对C2C12细胞分化的多细胞核融合的肌管数目的影响

Fig.5 The effects of continuous single-dose ISO on the number of myotube nuclear fusion during C2C12 cells differentiation



A: Western blot检测分析连续单次给不同剂量(10^{-8} mol/L、10^{-7} mol/L、10^{-6} mol/L 、10^{-5} mol/L)ISO处理后肌细胞生成蛋白的变化; B、C: 半定量分析显示MYH和MyoG表达变化, n=3, *P<0.05。

A: the levels of MyoG in C2C12 cells were detected in response to continuous single-dose ISO (10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L, 10^{-5} mol/L) by Western blot; B,C: semi-quantitative analysis showed traits of MYH and MyoG levels, n=3, *P<0.05.

图6 连续单次给予ISO抑制了C2C12分化的骨骼肌细胞中肌细胞生成蛋白的表达

Fig.6 Continuous single-dose ISO administration inhibited the expressions of MyoG in skeletal muscle cells differentiated from C2C12 cells

2.4 连续单次ISO在C2C12细胞分化过程中对肌球蛋白重链和肌细胞生成蛋白水平的影响

Western blot结果显示, 在连续单次给予ISO作用下, 随着剂量的增加, MYH水平逐渐降低, 且在10^{-5} mol/L ISO时达到最低。与此同时, 发现肌细胞生成蛋白(MyoG)水平也随着ISO浓度的增多而下降, 也在10^{-5} mol/L ISO时达到最低(图6)。

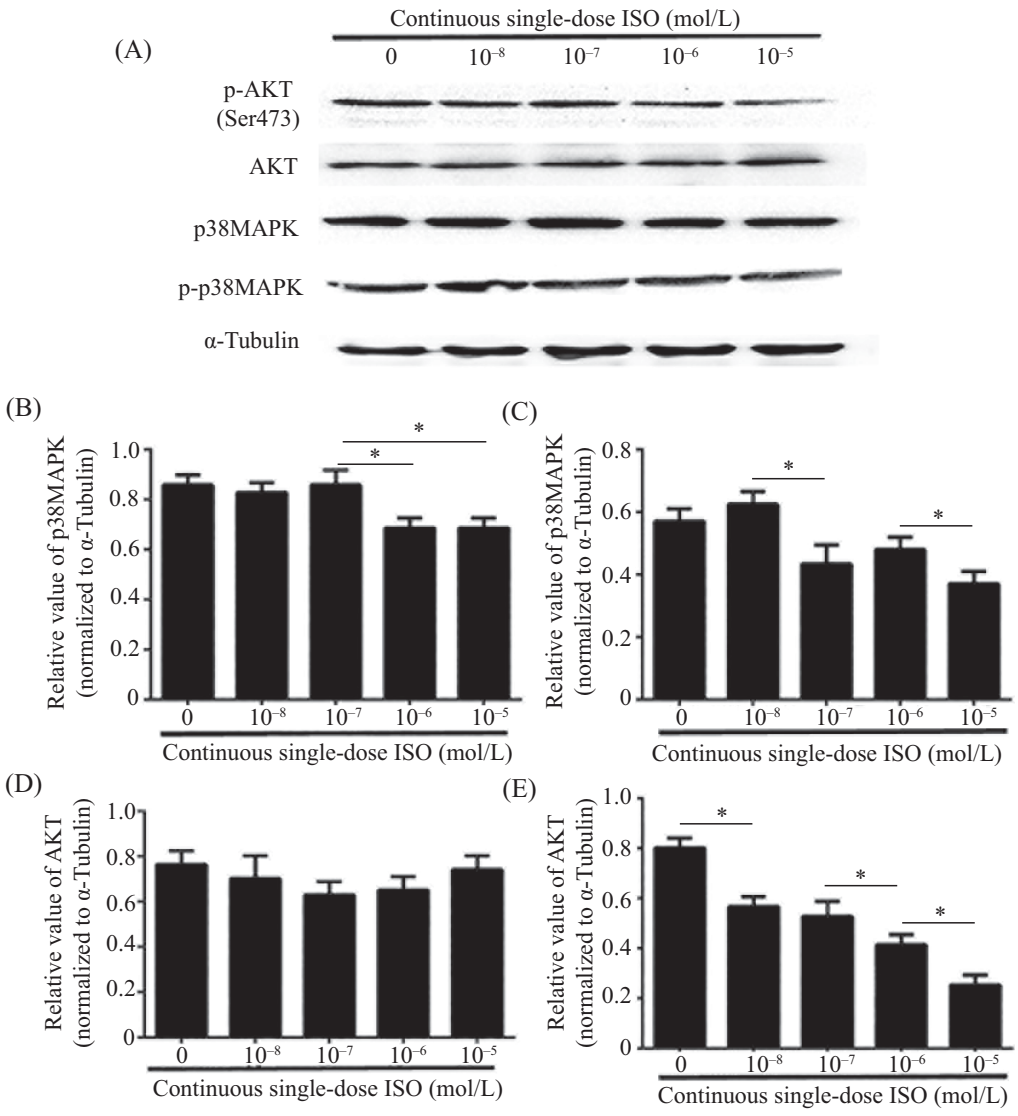
2.5 连续单次给予ISO在C2C12细胞分化过程中对p38MAPK和AKT信号分子的影响

Western blot结果显示, 在连续单次给予ISO作用下, 随着剂量的增加, p38MAPK和AKT水平变化不明显。但是, p-p38MAPK和p-AKT的水平随着ISO浓度的增加而逐渐降低, 且在10^{-5} mol/L ISO时达到最低(图7)。

3 讨论

心力衰竭是多种心血管疾病的严重和终末阶段, 发生率、致残率和居高不下, 是影响国人健康的主要原因之一, 具有重大的疾病负担和社会负担。心衰常呈现出自主神经功能障碍(交感神经兴奋增加或副交感神经兴奋减少)的特征[4-5], 除了与恶性心律失常及其他症状的发生密切相关外[11], 还与通过氧化应激及炎症等介导的心肌和骨骼肌的萎缩息息相关[8-10]。本研究不仅建立了一个体外模拟交感神经长期过度兴奋的骨骼肌分化失衡模型, 还发现骨骼肌分化失衡与其造成的分化调节因子表达降低有关。

由于交感神经过度兴奋常以去甲肾上腺素(norepinephrine, NE)升高所造成的损伤为主, 且这种



A: Western blot检测分析连续单次给不同剂量(10^{-8} mol/L、10^{-7} mol/L、10^{-6} mol/L 、10^{-5} mol/L)ISO处理后p38MAPK、AKT、p-p38MAPK和p-AKT的变化; B~E: 半定量分析p38MAPK、AKT、p-p38MAPK和p-AKT的水平, n=3, *P<0.05。

A: the levels of p38MAPK, AKT, p-p38MAPK and p-AKT in differentiated C2C12 cells were detected in response to continuous single-dose ISO (10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L, 10^{-5} mol/L) by Western blot; B-E: semi-quantitative analysis were performed as indicated proteins, n=3, *P<0.05.

图7 连续单次给予ISO通过降低p-p38MAPK和p-AKT抑制了C2C12细胞分化

Fig.7 Continuous single-dose ISO administration inhibited C2C12 cells differentiation by decreasing levels of p-p38MAPK and p-AKT

损伤以结合β-AdR为主[7]。除了体外易被氧化不够稳定外, NE即可与β-AdR结合, 也可与α-AdR结合, 而NE与不同的受体结合后激活信号转导机制及其相伴随的生物学效应存在很大差异, 这些差异为具体分析NE的作用及机制带来不便[2]。因此, 国内外学者大多采用更为稳定且主要与β-AdR结合的异丙肾上腺素(ISO)干预来模拟交感神经过度兴奋的效应。在以往的研究中, 国内外学者大多采用单独一次给予ISO的方式, 且ISO浓度是5×10^{-5} mol/L, 甚至10^{-4} mol/L, 方可复制交感神经过度兴奋时骨骼肌萎缩的效应[12-13]。在本研究中, 利用国内外通用的C2C12细胞作为材料, 建立了C2C12细胞分化成熟骨骼肌细胞的分化模型, 发现C2C12细胞呈现出肾上腺素能受体表达的特征, 暗示交感神经过度兴奋时可能通过结合其受体而发挥调节C2C12细胞分化的作用。因此, 我们在分化模型的基础上, 单次给予高剂量ISO, 发现能够抑制C2C12细胞的分化, 但是没有连续单次给予ISO抑制效果明显。而且, 连

续单次给予不同浓度的ISO处理后, C2C12细胞分化形成多细胞核融合肌管的能力逐渐降低, 这与临床长期的交感神经过度兴奋造成骨骼肌萎缩的临床表现一致[6]。可见, 连续单次给予ISO复制在体交感神经过度兴奋造成骨骼肌萎缩的模型更接近临床, 且更有临床意义。

为研究C2C12细胞分化形成肌管潜在的机制, 我们分析了ISO对肌细胞生成蛋白(MyoG)水平的影响, 发现ISO随着浓度的增加MyoG的水平逐渐降低, 即呈现浓度依赖的特征, 暗示其作用与MyoG有关。实际上, 以往的研究显示, *MyoG*基因是骨骼肌的生肌决定因子, 其特征是不仅在所有骨骼肌细胞系中均可检测到, 而且是骨骼肌成肌细胞分化为成熟骨骼肌细胞所必需的调控因子。更重要的是, MyoG通过控制骨骼肌成肌细胞的融合和肌纤维的形成在C2C12细胞分化中起关键作用[14]。因此, MyoG在骨骼肌成肌细胞分化为成熟骨骼肌细胞中的作用不仅是唯一且不可替代的[15]。本研究发现, 连续单次给予ISO后, 肌管细胞核融合数目逐渐减少, 呈现出ISO浓度依赖的特征, 表明ISO抑制了C2C12细胞的分化。在此基础上分析MyoG的水平变化特征与肌管形成特征, 发现二者之间具有密切关联, 提示肌管形成与MyoG水平变化有关。上述这些变化与在体交感神经长期过度兴奋所致的心肌和骨骼肌分化抑制是一致的[16-17]。

已有的研究提示, p-p38MAPK和p-AKT在C2C12细胞分化过程中发挥关键作用[18-20], 其水平增加与C2C12细胞分化为成熟的骨骼肌细胞息息相关。为此, 我们检测了连续单次给予ISO后, C2C12细胞p-p38MAPK和p-AKT的水平变化, 发现p-p38MAPK和p-AKT水平降低, 这与以往报道的p-p38MAPK和p-AKT介导C2C12细胞分化的研究结果一致。但是其具体关联机制还需要进一步探索。总之, 阐明交感神经长期过度兴奋抑制成肌细胞分化的机制将为临床治疗心衰相关联的心肌及骨骼肌的萎缩和无力的防治提供新的依据。

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