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论著

丹参酮IIA对新生大鼠缺氧缺血性脑损伤磷酸化NMDA受体1表达及细胞内游离钙浓度的影响

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摘要：

目的：观察丹参酮ⅡA（TanIIA）对新生大鼠缺氧缺血性脑损伤（HIBD）脑皮质神经细胞S897位点磷酸化的NMDA受体-1亚基（phospho-NR1 S897）表达以及细胞内游离钙离子浓度（［Ca2+］i）的影响，探讨TanIIA对HIBD的保护作用机制。方法：7日龄新生SD大鼠240只随机分为正常对照组、HIBD组及HIBD+TanIIA组，按Rice-Vannucci方法制作HIBD动物模型，时间点选择HIDB后3, 6, 12和24 h（各组各时间点n=10）。TanIIA按1 μg/g每12 h腹腔注射1次。应用Fura-2 AM 标记和日立F-4500型荧光扫描仪测定［Ca2+］i。应用免疫荧光组化染色方法测定phospho-NR1 S897的表达。结果：（1）与正常对照组比较，HIBD组损伤侧脑细胞［Ca2+］i绝对值和损伤侧与对侧的［Ca2+］i比值明显升高，各时间点的差异均具有统计学意义（P<0.05）；与HIBD组比较，HIBD+ TanIIA组各时间点的［Ca2+］i升高的程度均有所减轻，其中24 h 损伤侧与对侧的［Ca2+］i比值较HIBD 组降低24.9 %，差异具有统计学意义（P<0.05）。（2）正常对照组大脑皮质有大量均匀分布的phospho-NR1 S897 染色阳性细胞。与正常对照组比较，HIBD组各时间点损伤侧脑皮质区phospho-NR1 S897阳性表达细胞数和绿色荧光强度均明显降低，差异均具有统计学意义（P<0.05）。与HIBD组比较，HIBD+ TanIIA组各时间点的损伤侧脑皮质区phospho-NR1 S897阳性表达细胞数和绿色荧光强度均升高，其中HIBD后3 h和24 h的差异具有统计学意义（P<0.05）。结论：TanIIA可以减轻HIBD导致的脑皮质区phospho-NR1 S897表达减弱的程度，并降低HIBD导致的［Ca2+］i升高的程度，提示TanIIA对脑细胞的保护作用可能是通过影响NMDA受体的表达，减少细胞内游离钙聚集而达到的。

关键词： 丹参酮IIA 缺氧缺血 脑损伤 phospho-NR1 S897 大鼠

Effect of Tanshinone IIA on phosphorylated NMDA receptor 1 expression and intracellular free calcium concentration in neonatal SD rats with hypoxic ischemic brain damage

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Abstract:

ObjectiveTo determine the effect of Tanshinone IIA (TanIIA) on the phosphory-lated NMDA receptor 1 at Serine 897 site (phospho-NR1 S897) and intracellular free calcium concentration (［Ca2+］i) in neonatal SD rats with hypoxic ischemic brain damage (HIBD), and to explore the neuroprotective mechanism of TanIIA in HIBD. MethodsNeonatal SD rats were randomly divided into a normal control, and an HIBD and TanIIA+HIBD group. Rice-Vannucci method was used for HIBD animal model. Time points were: 3, 6, 12, and 24 h after HIBD (n=10 in each group at each time point). TanIIA was intraperitoneally given at 1 μg/g every 12 h. Fura-2AM was used to mark the fluorescent calcium probe and ［Ca2+］i was measured by a Hitachi F-4500 Fluorescence Spectrophometer. Fluorescent immunohisotichemical study was used for the expression of phospho-NR1 S897.Results(1) Compared with the normal control group, both the ［Ca2+］i absolute number and ipsi-/contra-lateral ratio were increased at each time point with statistical significance (P<0.05). Compared with the HIBD group, the ［Ca2+］i in the HIBD+ TanIIA group was decreased at each time point. At 24 h after HIBD, the ipsi-/contra-lateral ratio of HIBD+ TanIIA group was 24.9% less than that of HIBD group with statistical significance (P<0.05). (2) In the normal control group, abundant phospho-NR1 S897 positive cells were nicely distributed in the cortex. Compared with the normal control group, at each time point, both the absolute number of phospho-NR1 S897 positive cells and the fluorescent intensity of phospho-NR1 S897 in the ipsilateral cortex of the HIBD group were decreased with statistical significance (P<0.05). Compared with the HIBD group, both the absolute number of phospho-NR1 S897 positive cells and the fluorescent intensity of phospho-NR1 S897 in the ipsilateral cortex of HIBD+ TanIIA were increased. There was significant difference at 3 and 12 h after the HIBD (P<0.05). ConclusionTanIIA reduced the HIBD-caused down-regulation of phospho-NR1 S897 and the HIBD-caused ［Ca2+］i elevation in the cortex. The neuroprotective effect of TanIIA may be related to influencing NMDA receptor expression and decreasing intracellular free calcium aggregation.

Keywords: Tanshinone IIA; hypoxia-ischemia; brain damage; phospho-NR1 S897; rat

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