目的：研究静注免疫球蛋白(IVIG)产生抗补体活性(ACA)的原因. **方法**：用二种方法测定ACA活性，高效液相色谱测定IgG多聚体、双聚体、单体及碎片组成，环状免疫扩散法测定IgG亚类和IgA含量，显色反应测定前激肽释放酶激活因子(PKA)和激肽释放酶(KK)活性. 酶联免疫吸附试验测定针对乙肝表面抗原的特异抗体(Anti-HBs). **结果**：二种ACA方法有相关性，但ACA活性与各种IgG分子组成、IgG亚类和IgA含量、PKA和KK无关. IVIG热培养后ACA活性显著升高，Anti-HBs活性则明显下降，而各种IgG分子组成则基本未变. **结论**：IgG分子结构变化增强IVIG自发抗补体活性.

关键词 静脉注射免疫球蛋白；补体激活；免疫球蛋白G；前激肽释放酶；激肽释放酶；多聚体

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Effect of ginsenosides against anoxic damage of hippocampal neurons in culture[1]

WANG Fu-Zhuang, DING Ai-Shi, LIU Zhen-Wei (*Department of Neurobiology, Institute of Basic Medical Sciences, Academy of Military Medical Sciences, Beijing 100850, China*)

AIM: To study the protective effect of ginsenosides (Gin) against anoxic injury in brain cells. **METHODS**: On d 12 after plating of the hippocampal cells from newborn rat, the cultures were exposed to anoxic atmosphere (95 % N_2+5 % CO_2) for 4—24 h. The cellular morphology, survival rate, and effluxes of lactate dehydrogenase (LDH) and K^+ from cells were observed. **RESULTS**: After 24-h anoxia, the survival rate of cells was decreased from 92 %±4 % before anoxia to 1.0 %±2.0 %; the effluxes of LDH and K^+ were increased from 2.3±0.6 U L^{-1} and 5.56 ±0.16 mmol L^{-1} before anoxia to 36±5 U L^{-1} and 8.5±0.7 mmol L^{-1}, respectively. In the Gin group exposed anoxia for 24 h, the survival rate of cells was 4 %±4 %; the effluxes of LDH and K^+ were 30±3 U L^{-1} and 7.9±0.8 mmol L^{-1}, respectively. All these changes were lower in Gin group than those of control. **CONCLUSION**: Under anoxia the cultured hippocampal neurons were seriously damaged, which may be protected by Gin.

KEY WORDS ginseng; saponins; anoxia; hippocampus; cultured cells

Ginsenosides (Gin), the main active component of ginseng, potentiated the nerve growth facter-mediated nerve fiber production in organ cultures of chicken embryonic dorsal root ganglia and sympathetic ganglia[1]. The crude saponin fraction of ginseng root had a proliferative effect on neurite extension of primary cultured neurons of the rat cerebral cortex[2]. The Gin has the protective effect against acute cerebral ischemia/reperfusion injury in rats[3]. Our previous data showed that the Gin delayed the onset of irreversible

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anoxic damage, markedly improved the recovery of the population spike, and had protective effect against acute hypoxia of rat hippocampal slices[4]. In the present paper we studied the protective effect of Gin against acute anoxic injury of hippocampal cells.

MATERIALS AND METHODS

Gin (ginseng stem-leaves saponins, Kuandian Pharmaceutical Factory, Dandong) was dissolved in Eagle's MEM (10 g L^{-1}) and filtered.

Cell cultures[5] Hippocampus was isolated from 1-d Wistar rats and incubated at 36 ℃ in 0.25 % trypsin for 30 min. The tissue was dissociated by gentle passage through the tip of a flame-narrowed Pasteur pipette, plated in 35-mm collagen-coated dishes (Falon) at a density of 1×10^5 cell L^{-1} and cultured at 36 ℃ in 5 % CO_2+95 % air. Medium contained 94 % Eagle's MEM, 5 % horse serum and 1 % nutrient supplement[6]. On d 3, 5'-fluoro-2'-deoxyuridine 15 mg L^{-1} and uridine 35 mg L^{-1} were added to inhibit further growth of the non-neuronal cells. Cultures were fed twice a week by replacing 50 % medium with fresh medium.

Immunocytochemistry Hippocampal cells on d 12 were immunostained using the antiserum of neuron-specific enolase (NSE) according to method of ABC (Vectastain ABC elite Kit, Vector Laboratories). Under a phase-contrast microscope 100 cells were counted.

Anoxia Hippocampal cells on d 12 were divided into control and Gin group. In Gin group cells were pretreated with Gin for 24 h before anoxia. The final concentration of Gin was 100 mg L^{-1}. The control was added with equal volume of Eagle's MEM. Anoxia was carried out with 95 % N_2+5 % CO_2 in a humidified atmosphere at 36 ℃ for 4, 8, 12, and 24 h. Viable cells were counted with phase-contrast microscopy (Japan, NIKON) from 50 microscopic fields (magnification × 200) after Trypan blue stain (0.4 %, 3 min).

Determination of lactate dehydrogenase (LDH) and K^+ After anoxia for 4 — 24 h, media samples were pooled for each group LDH and K^+ were determined by autoanalyzer (Impact 400, United Gilford Co) and flame photometer (Flame-30c, Japan Spectrophoto).

Statistics Statistical analysis was done using *t* test for differences between control and Gin groups.

RESULTS

Morphology of 12-d old cultured hippocampal cells, most neurons were pyramidal- or polypolar-shaped with high refractile and stereognostic, interconnected by a rich fiber network. Immunohistochemically for enolase, NSE-positive neurons and fine dendrites were identified clearly. The stained neurons comprised more than 90 % of total cells (Fig 1, Plate 1).

Effects of anoxia The swollen neurons at 4 and 8 h were 18 % and 85 % of total cells, respectively. The refraction was lost in part of neurons and some of them became vacuolated (Fig 1C). After 12-h anoxia, most neuronal membranes were ruptured, processes were disintegrated, and finally cells died (Fig 1E). Almost all neurons were dead after anoxia for 24 h (Tab 1).

The effluxes of LDH and K^+ into the bathing medium were gradually increased (Tab 1).

Protective effect of Gin Most neurons incubated with Gin maintained original morphology when exposed to anoxia for 8 h. A distinct glow surrounding the neuronal body, nucleus, and nucleolus was seen clearly. At 4 and 8 h about 6 % and 43 % of total cells became swollen, respectively (Fig 1D). The neuronal damage induced by anoxia for 12 h in Gin group was less than that in control (Fig 1F). In the Gin group, the survival rate of cells was higher than that in control group (Tab 1). In Gin group, cellular effluxes of LDH and K^+ induced by anoxia of 4 — 24 h were lower than those in control group (Tab 1).

Tab 1. LDH, K^+ efflux, and survival of hippocampal neurons after anoxia. $n=5$ for LDH and K^+ efflux, $n=500$ for survival. $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

Groups	Before anoxia	After anoxia 4 h	8 h	12 h	24 h
LDH (U L^{-1})					
Control	2.3±0.6	14.5±0.8	19.2±0.8	26.7±1.2	36±5
	2.4±0.4	11.4±1.1[c]	16.8±1.4[c]	23.5±1.2[c]	30±3[b]
K^+ (mmol L^{-1})					
Control	5.56±0.16	6.00±0.14	6.68±0.23	7.7±0.3	8.5±0.7
Gin	5.57±0.11	5.72±0.13[b]	6.10±0.10[c]	7.0±0.3[b]	7.9±0.8[b]
Survival (%)					
Control	92±4	83.3±2.7	55±6	23±5	1.0±2.0
Gin	92±3	87±4[c]	58±6[b]	27±6[c]	4±4[c]

DISCUSSION

In the present study we developed the dissociated cell cultures of hippocampal neurons from new born rats using split inhibitor to suppress proliferation of non-neuronal cells. From the NSE immunocytochemistry, it proved that the neurons occupied more than 90 % in cultures of 12 d, and considered that it was relatively pure neuronal cultures. Our results showed that the cultured hippocampal neurons were very sensitive to anoxia and the morphological changes induced by anoxia were similar to those observation on anoxic damage of embryonic hippocampal neurons[7]. These results also proved that anoxic damage of hippocampal neurons from newborn rat was very severe and more serious as the time of anoxia went on. Whereas the morphological changes of cultured neuron pretreated with Gin were improved and the number of survival cells was higher than those of anoxic control. These findings directly proved that Gin had protective effect on hippocampal cells during anoxia.

As a stable enzyme marker, LDH is plentiful in central nervous system. Under normal conditions, the efflux of LDH from neurons are very low, but it was obviously increased after anoxic damage[8]. In our experiment on the efflux of LDH and K^+, the results measured were similar to Sher's report[9]. With the prolongation of anoxic time, the increases of LDH and K^+ effluxes were corresponding to the loss of surviving cells in culture, which indicated that the permeability of the membrane of hippocampal neurons was increased and the severe damage of cerebral cells did exist. The elevation of extracellular K^+ during anoxia was correlated to anoxic membrane depolarization of neurons[10]. For the effluxes of LDH and K^+ were suppressed in Gin-treated cells, it seems that Gin can protect neuronal membrane against anoxic damage. These findings also confirmed that the protection of ginsenosides on post-anoxic recovery of synaptic transmission[4] was associated with decreased K^+ efflux, preventing from anoxic depolarization in hippocampal neurons.

In summary, the present results showed that the Gin can attenuate the morphological changes of hippocampal neurons induced by anoxia, improve the cell survival and reduce the effluxes of LDH and K^+ during anoxia. It suggests that the Gin exerts direct protective

effect on cerebral cells during anoxia. As to the underlying mechanisms of this protection, further investigation would be needed.

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人参皂苷抗海马培养细胞缺氧损伤的作用[1]

王福庄，丁爱石，刘振伟（军事医学科学院基础医学研究所神经生物室，北京100850，中国）

目的：观察人参皂苷抗脑细胞缺氧损伤的作用. **方法**：将12 d 培龄的海马细胞置于95 % N_2+5 % CO_2中4—24 h，比较人参皂苷组和对照组细胞形态学. 存活率及 LDH 和 K^+流出的变化. **结果**：缺氧24 h 后，对照细胞存活率从缺氧前92 %±4 %降至1.0 %±2.0 %；LDH 和 K^+漏出量分别由2.3±0.6 U L^{-1}和5.56±0.16 mmol L^{-1}增至36±5 U L^{-1}和8.5±0.7 mmol L^{-1}；此时，人参皂苷组细胞存活率为4 %±4 %；LDH 漏出量为30±3 U L^{-1}，K^-含量为 7.9±0.8 mmol L^{-1}. 与对照组相比，人参皂苷组受损程度明显减轻. **结论**：人参皂苷具有抗海马细胞缺氧损伤的作用.

关键词 人参；皂苷类；缺氧症；海马；培养的细胞

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