大改进。但我们进一步研究结果表明,如将采血量增至 6 ml,则可明显提高 CEC收集数量和培养成功率。我们还认为,分离 CEC的重要环节是将 Percoll分离液密度标化为 1.050,否则,在分离的细胞中会混有较多量的其它细胞,遭致培养失败。本研究属此种情况仅见一例。

3.4 培养技术

本研究尚未遇及由于培养技术的原因而造成培养失败的问题。但必须强调,在培养 CEC的过程中要很好掌握细胞数调整、培养液配制、二氧化碳浓度和换液等重要环节,方能保证培养成功。

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In vitro culture of circulating endothelial cells and its influential factors after severe multiple trauma

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Abstract Objective: To explore *in vitro* culture of circulating endothelial cells (CECs) and its influential factors after severe multiple trauma. Methods: After being isolated from the peripheral blood in 17 patients with severe multiple trauma (ISS≥ 20), the CECs were differentiated and then cultured *in vitro*. Meanwhile, the influential factors on the culture were observed and analyzed. Results: The total successful culturing rate was 62.5%. The severer the trauma was, the higher the successful culturing rate was. The influential factors on the culture were severity of trauma, time for blood sampling and techniques of isolation and culture. Conclusion: In this study, the successful rate of in vitro culture of CECs was increased through improving the techniques of isolation and culture and overcoming the unfavorable influential factors. This provides a new method for further study on the surface components of CECs.

Key words multiple trauma; circulating endothelial cell; *in vitro* culture

海马神经化学因素对兔 P3波影响机制的实验研究

An experimental study on hippocampal neurochemical mechanisms responsible for P3 potential changes in rabbits

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迄今,P3波电位作为辅助诊断精神、神经性等认知功能损害性疾病、评估病程、疗效及预后的重要指标,为临床提供了有价值的客观定量无创性电生理检测手段,是对既往临床诊治精神、心理性疾病过份依赖于现象描述等行为学指标的可靠补充。P3波的发生是脑内各种神经化学因素,如递质、调质等在不同脑区协同活动的结果。神经化学因素的失衡必然导致电位异

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Effects of insulin on proliferation of human vascular smooth muscle cells

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Abstract Objective: To investigate the effects of insulin on proliferation of human mesenteric arterial smooth muscle cells (ASMCs). Methods: After the primary culture of human ASMCs was successfully performed with primary-explant method, it was interfered with insulin. Then the proliferation of ASMCs was detected with MTT micro-enzymatic determination. Results: The proliferation of ASMCs was significantly increased. The cytoflowmetry showed that more ASMCs in insulin-and PMA-interfered groups got into the S and G_2+ M periods as compared to the control. Conclusion: Insulin can promote the proliferation of human ASMCs and the growth of ASMCs depends on the insulin concentration.

Key words human arterial smooth muscle cell; insulin; atherosclerosis; proliferation

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常。显然,相应机制的研究与阐明,可为认知功能紊乱性疾病的诊断、治疗及病因揭示奠定基础。目前,神经化学因素对 P3波的影响机制尚不清楚,相关研究较少。为此,本题在已建立 Oddball方案被动条件记录听诱发兔 P3波研究模型基础上,针对与 P3波起源、认知功能紊乱性疾病的发生密切相关的海马结构,从受体亚型、递质、细胞与分子水平探讨该结构内 NMDAR GABAAR GABABR活动及病理条件下 Glu, GABA, NO等变化对兔 P3波的影响机制。

主要方法、结果与结论如下:

(1)采用海马 CA1区微量注入 GABAAR选择性拮抗剂 Bicuculline, GABABR激动剂 BacLofen,观察 GABA受体亚型对兔 P3波的作用。结果发现: Bicuculline(3.6.12 umol/L)呈一定量效依赖衰减 P3b波电压振幅, Baclofen(6.25 12.5 25 mmol/L)呈明显量效依赖延长、阻抑 P3b波。外源性 GABA (100 mmol/L)可翻转 Bicuculline(12 umol/L)、Baclofen(25 mmol/L)对 P3b波振幅的抑制,但其潜伏期延长。结果提示:海马 CA1区 GABAAR IPSP可能即是 P3b波振幅的重要组成。CA1区 GABABR的活动可通过减弱 P3b波发生相关神经元兴奋的易化,故主要延长其潜伏期,最终也可完全抑制 P3b波。外源性 GABA可通过增强 GABAAR IPSP使 P3b波电压振幅恢复,但同时作用于 GABABR等,又延长其潜伏期。

(2)采用 NMDAR竞争性拮抗剂 AP-5(3.125 6.25 12.5 mmol/L)在海马 CA1、CA3区微量注入,以推测 NMDAR EPSP活动对兔 P3波的作用。结果发现: AP-5在 CA1区呈一定量效依赖延长 P3a波潜伏期。AP-5在 CA1、CA3区为明显量效依赖延长 P3b波潜伏期。AP-5在 CA1、CA3区对 P3a、P3b波电压振幅无明显影响。结果提示: CA1区 NMDAR EPSP活动是 P3a波发生相关神经元兴奋的易化因素之一,可能是海马实施对 P3a波潜伏期调节的重要机制。CA1、CA3区 NMDAR EPSP可能共同对 P3b波发生相关神经元兴奋起易化作用,故可影响其潜伏期。

(3)隔区损毁后不同时相点记录兔 P3波电位,随后用 BecKman-6200型高效氨基酸测定仪分析相应海马 Glu, GABA, Asp, Tau含量,并结合分析相关性,研究这些递质变化在 P3波异常中的意义。结果表明:隔损毁后 P3a、P3b波潜伏期、电压振幅分别呈不同程度的延长、衰减。海马 Glu逐渐降低,并与 P3a、P3b波潜伏期延长呈一定意义负相关。GABA下降则与 P3b波振幅变化呈一定意义正相关。在本实验隔损毁后,海马 Asp、Tau无明显变化。结果提示:隔损毁后,海马兴奋性氨基酸 Glu降低,可能通过减弱 P3a、P3b波发生相关神经元的易化与激活使其潜伏期延长。海马抑制性 GABA下降,其相应 IPSP活动减弱可能是 P3b波振幅衰减的原因。海马 Asp、Tau可能未参与隔损毁后兔 P3波损害过程。

(4)隔损毁后不同时相点记录兔 P3波电位,以 bcNOSmRNA原位杂交、NADPH-d组化、Nissl染色技术,结合药物干预手段,观察海马 bcNOS异常表达,推测在此条件下 NO对 P3波可能的影响。结果显示:隔损毁后,海马 bcNOSmRNA、NADPH-d表达逐渐增强、增加,乃至 NADPH-d阳性神经元自身结构紊乱及海马 CA1、CA2区锥体细胞 Nissl体大量溶解、消失。这一病理过程可能与 P3b波的消失有关。在连续应用低剂量非选择性 NOS抑制剂 L-NAME后,可干预上述病理过程,使 P3b波消失延缓。结果提示:隔损毁后诱发海马 bcNOS表达的动态增多、增高,其过量产生的 NO致使 bcNOS神经元自身和海马 CA1、CA2区锥体细胞选择性损伤,该病理过程可能损害 P3b波电位发生的最后环节,可能是隔损毁后 P3b波消失的重要机制。

关键词　P3;海马;递质; NMDAR; GABAAR; GABABR; Glu GABA

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