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脉冲电流经皮刺激肝区对运动性疲劳大鼠 5-HT 及其代谢的影响 *

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摘要 目的: 研究脉冲电流经皮刺激肝区对运动性疲劳大鼠大脑纹状体 5-羟色胺(5-HT)及其代谢的影响。**方法:** 8 周龄 Wistar 雄性大鼠 80 只随机分为安静对照组(CG 组)、疲劳训练组(FG 组)、运动后刺激组(SAF 组)、运动前刺激组(SBF 组),除 CG 组外各组均进行游泳训练,建立运动疲劳模型,于第 1、3、5 周训练的最后 1d,断头处死,取外周血测游离色氨酸(F-Trp)、支链氨基酸(BCAA)、F-Trp/BCAA 含量,取纹状体测 F-Trp、5-HT、5-羟吲哚乙酸(5-HIAA)含量。**结果:** 与 CG 组相比,各组大鼠血清 BCAA 含量呈下降趋势,其余各指标均呈上升趋势;与 FG 组相比,SAF 组、SBF 组第 3 周末纹状体 5-HT 明显下降($P < 0.01$);与 SBF 组相比,SAF 组第 5 周末纹状体 5-HT 明显下降($P < 0.05$)。**结论:** 经皮脉冲电流刺激具有降低疲劳大鼠 5-HT 及代谢产物的含量,有助于疲劳的消除,对长时间运动时中枢机能的改善具有积极作用。

关键词: 脉冲电流;中枢疲劳;纹状体;5-羟色胺

中图分类号: Q95-3; Q68; R333.4 **文献标识码:** A **文章编号:** 1673-6273(2015)17-3259-05

Influence of Percutaneous Impulsive Current Stimulation of Hepatic Region on the Concentration of 5-HT and Its Metabolism in Exercise-induced Fatigue Rats*

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ABSTRACT Objective: To investigate the effects of percutaneous impulsive current stimulation of hepatic region on the concentration of 5-hydroxytryptamine (5-HT) and its metabolism in striatum of fatigued rats. **Methods:** 80 Eight-week-year old Wistar male rats were randomly divided into four groups: control group (CG), fatigue group (FG), stimulation after fatigue group (SAF) and stimulation before fatigue group (SBF). All of the groups were given the swimming training to establish exercise-induced fatigue model except for the CG group. Rats were killed immediately, The level of free-tryptophan(F-Trp), branched chain amino aci(BCAA) and the ratios of F-Trp/BCAA were Measured in blood serum of rats, the concentration of F-Trp, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were tested in Striatum of Rats. **Results:** Compared with CG group, the concentrations of BCAA in blood serum of rats in every group tended to decline, other indexes were all increased. Compared with FG group, the concentrations of 5-HT in rats' striatum in SAF group and SBF group declined significantly($P < 0.01$) at the weekend of 3rd week. Compared with SBF group, the concentrations of 5-HT in rats' striatum in SAF group declined significantly ($P < 0.01$) at the weekend of 5th week. **Conclusions:** Percutaneous impulsive current stimulation of hepatic region can effectively lessen the concentration of 5-HT and its metabolism in fatigued rats, which could suspend the occurrence of fatigue and help to eliminate the fatigue, improve rat central nerves performance in long time exhausted exercise.

Key words: Impulsive current; Central nervous system fatigue; Striatum; 5-hydroxytryptamine

Chinese Library Classification(CLC): Q95-3; Q68; R333.4 **Document code:** A

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前言

运动性疲劳(Sports fatigue)是指机体的生理过程不能持续其机能在一特定水平或不能维持预定的运动强度,运动性疲劳的防治研究一直是军事医学和运动医学领域研究的热点问题。

NOAKES^[1]研究认为,疲劳是一种源于大脑的用来调控运动行为的情绪,以确保整个机体的自稳态,提出“中枢管理模式”(central governor model)学说。长时间运动过程中纹状体神经元参与运动疲劳的中枢调控^[2],而在中枢神经系统内富含 5-HT 神经元的脑中神经纤维可投射到纹状体和黑质等部位^[3],研究发

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现,急性力竭性运动使大鼠纹状体 5-HT 及其代谢产物显著上升^[4]。全军军事训练医学研究所研究发现,中频脉冲电流经皮刺激疲劳大鼠肝区可缓解外周疲劳^[5]。本实验在前期研究的基础上,探讨经皮脉冲电流刺激肝区对疲劳大鼠 5-HT 及其代谢的影响。

1 材料与方法

1.1 实验动物

清洁级雄性 8 周龄 Wistar 大鼠 80 只,体重 204 ± 15 g,由河南省实验动物中心提供,许可证号 SCXK(豫)2013-0004,饲养室温度 23 ± 2 °C,湿度 $41\% \pm 15\%$,自然光照,分笼饲养,自由摄食及饮水。

1.2 主要试剂和仪器

大鼠血清 F-Trp、BCAA 酶联免疫分析试剂盒,大鼠纹状体 F-Trp、5-HT、5-HIAA 酶联免疫分析试剂盒(上海江莱生物科技有限公司提供,德国 IBL 产品),严格按照使用说明操作。用酶标仪在 450 nm 波长测试吸光度(OD 值),测定血清 F-Trp、BCAA、纹状体 F-Trp、5-HT 和 5-HIAA 浓度。酶标仪(美国伯乐 BIO-RAD)、超声匀浆机(宁波新艺 SCIENTZ)、离心机(北京雷勃尔)。

1.3 实验方法

1.3.1 动物模型建立及分组 将实验动物随机分为 4 组:安静对照组(CG 组)、疲劳训练组(FG 组)、运动后刺激组(SAF 组)、运动前刺激组(SBF 组),每组 18 只,剩余备用。CG 组不进行任何训练,FG 组、SAF 组及 SBF 组大鼠连续 5 周进行游泳训练,水深 60 cm,水温(31 ± 2) °C,每周训练 6 d,休息 1 d,游泳 2 次/

日,每次均达到力竭,当大鼠浮在水面不运动时用玻璃棒驱赶,维持其运动状态,力竭标准以大鼠下沉后 10 s 不能游回水面。SAF 组大鼠游泳力竭后,20%乌拉坦以 1 mL / 200 g 体重^[6]进行皮下注射麻醉,给予经皮肝区脉冲电流刺激,SBF 组游泳前,将大鼠在固定装置上给予经皮肝区脉冲电流刺激,脉冲电流刺激频率为 1024 Hz,电流强度为 10 mA,间动周期为 1 s,时间为 20 min。

1.3.2 取材、标本制备及检测指标 各组大鼠分别在 1、3、5 周训练的最后 1 d,断头处死,取纹状体测游离色氨酸(F-Trp)、5-HT、5-羟吲哚乙酸(5-HIAA)含量,取血清测游离色氨酸(F-Trp)、支链氨基酸(BCAA)、F-Trp/BCAA 含量。

1.4 统计学分析

所有实验数据均用 SPSS18.0 统计学软件对实验数据进行统计学分析,数据用 $\bar{x} \pm s$ 表示,组间比较采用单因素方差分析,以 $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 经皮脉冲电流刺激疲劳大鼠肝区对血清 F-Trp、BCAA 含量的影响

第 1 周末四组大鼠血清 F-Trp、BCAA 变化不明显。第 3 周末血清 F-Trp 结果:FG 组、SBF 组与 CG 相比 $P < 0.01$,血清 BCAA 结果:FG 组、SAF 组、SBF 组与 CG 组相比 $P < 0.01$,SAF 组与 FG 组相比 $P < 0.05$ 。第 5 周末血清 F-Trp 结果:FG 组、SAF 组、SBF 组与 CG 组相比 $P < 0.01$,血清 BCAA 结果:SAF 组、SBF 组与 FG 组相比 $P < 0.01$ (表 1)。

表 1 各组大鼠血清 F-Trp、BCAA 含量的比较($\bar{x} \pm s, n=6$)

Table 1 The concentration of F-Trp and BCAA in blood serum of rats($\bar{x} \pm s, n=6$)

Item	CG group	FG group	SAF group	SBF group	F	P value
F-Trp($\mu\text{mol/L}$)						
1st weekend	38.61 ± 2.65	42.34 ± 3.87	40.27 ± 2.43	42.54 ± 4.21	1.829	0.174
3rd weekend	39.52 ± 2.27	$45.58 \pm 3.93^{(1)}$	41.59 ± 3.46	$44.37 \pm 3.42^{(1)}$	3.623	0.032
5th weekend	38.84 ± 3.49	$52.26 \pm 4.28^{(1)}$	$48.46 \pm 2.62^{(1)}$	$49.29 \pm 2.02^{(1)}$	17.376	0.000
BCAA($\mu\text{g/L}$)						
1st weekend	63.08 ± 3.96	58.39 ± 4.21	60.52 ± 3.71	62.76 ± 5.70	1.436	0.262
3rd weekend	64.16 ± 3.63	$50.77 \pm 4.92^{(1)}$	$57.18 \pm 3.56^{(1)(4)}$	$55.06 \pm 4.25^{(1)}$	11.006	0.000
5th weekend	62.68 ± 3.72	$39.78 \pm 4.22^{(1)}$	$50.56 \pm 2.94^{(1)(3)}$	$47.09 \pm 4.55^{(1)(3)}$	35.862	0.000

Note: (1) $P < 0.01$ Compared with CG group; (2) $P < 0.05$ Compared with CG group; (3) $P < 0.01$ Compared with FG group; (4) $P < 0.05$ Compared with FG group.

2.2 经皮脉冲电流刺激疲劳大鼠肝区对血清 F-Trp/BCAA 的影响

第 1 周末四组大鼠血清 F-Trp/BCAA 变化不明显。第 3、5 周末 FG 组、SAF 组、SBF 组与 CG 组相比明显升高 $P < 0.01$,第 3 周末 SAF 组与 FG 组相比明显下降 $P < 0.01$,SBF 组与 FG 相比下降 $P < 0.05$,第 5 周末 SAF 组与 FG 组相比明显下降 $P < 0.01$ (图 1)。

2.3 经皮脉冲电流刺激疲劳大鼠肝区对大鼠纹状体 F-Trp、5-HT、5-HIAA 含量的影响

F-Trp 结果:第 3 周末 FG 组与 CG 组相比明显升高 $P < 0.01$,

第 5 周末 FG 组、SBF 组与 CG 组相比明显升高 $P < 0.01$,SAF 组与 FG 组相比明显下降 $P < 0.01$ 。5-HT 结果:第 1、3、5 周末 FG 组、SAF 组、SBF 组与 CG 组相比明显升高 $P < 0.01$,第 3、5 周末 SAF 组与 FG 组相比明显降低 $P < 0.01$,SAF 组与 SBF 组相比明显降低 $P < 0.05$ 。5-HIAA 结果:第 3 周末 FG 组与 CG 组相比明显升高 $P < 0.01$,SAF 组与 FG 组相比明显降低 $P < 0.05$,第 5 周末 FG 组、SBF 组与 CG 组相比明显升高 $P < 0.01$,SAF 组、SBF 组与 FG 组相比 $P < 0.01$,SAF 组与 SBF 组相比明显降低 $P < 0.01$ 。

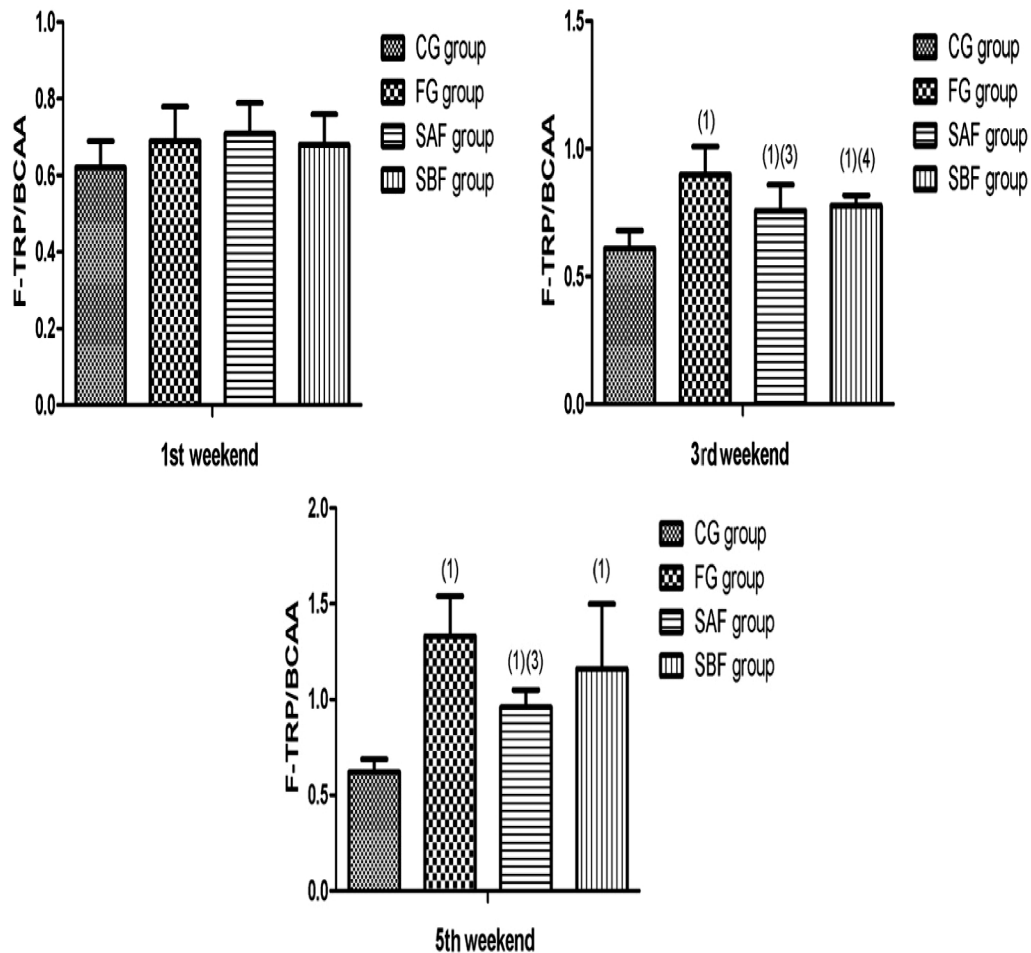


图1 各组大鼠血清 F-Trp/BCAA

Fig. 1 The ratios of F-Trp/BCAA in blood serum of rats

Note: (1) $P < 0.01$ compared with CG group; (2) $P < 0.05$ compared with CG group; (3) $P < 0.01$ compared with FG group; (4) $P < 0.05$ compared with FG group

表2 各组大鼠纹状体 F-Trp、5-HT、5-HIAA 含量的比较 ($\bar{x} \pm s$, $n=6$)Table 2 The concentration of F-Trp, 5-HT and 5-HIAA in striatum of rats every group ($\bar{x} \pm s$, $n=6$)

Item	CG group	FG group	SAF group	SBF group	F	P value
F-Trp($\mu\text{mol/L}$)						
1st weekend	21.56 $\pm$ 2.13	24.56 $\pm$ 2.13	20.37 $\pm$ 1.48	21.12 $\pm$ 1.76	2.698	0.073
3rd weekend	20.80 $\pm$ 2.96	26.62 $\pm$ 3.77 ⁽¹⁾	23.22 $\pm$ 3.58	23.33 $\pm$ 2.78	3.143	0.048
5th weekend	22.21 $\pm$ 2.84	32.08 $\pm$ 4.54 ⁽¹⁾	25.42 $\pm$ 4.70 ⁽³⁾	29.08 $\pm$ 3.38 ⁽¹⁾	7.131	0.002
5-HT($\mu\text{g/L}$)						
1st weekend	40.17 $\pm$ 2.85	70.62 $\pm$ 4.65 ⁽¹⁾	65.46 $\pm$ 5.27 ⁽¹⁾	68.81 $\pm$ 6.87 ⁽¹⁾	46.341	0.000
3rd weekend	41.34 $\pm$ 3.73	83.65 $\pm$ 4.39 ⁽¹⁾	66.47 $\pm$ 7.23 ⁽¹⁾⁽³⁾⁽⁶⁾	74.44 $\pm$ 4.26 ⁽¹⁾⁽³⁾	76.462	0.000
5th weekend	40.33 $\pm$ 3.07	77.58 $\pm$ 5.72 ⁽¹⁾	59.38 $\pm$ 4.51 ⁽¹⁾⁽³⁾⁽⁶⁾	90.41 $\pm$ 8.04 ⁽¹⁾⁽³⁾	89.919	0.001
5-HIAA ($\mu\text{g/L}$)						
1st weekend	1.65 $\pm$ 0.75	1.97 $\pm$ 0.37	1.80 $\pm$ 0.67	1.88 $\pm$ 0.70	0.275	0.843
3rd weekend	1.69 $\pm$ 0.65	3.51 $\pm$ 1.20 ⁽¹⁾	2.18 $\pm$ 0.45 ⁽⁴⁾	2.65 $\pm$ 0.90	5.018	0.009
5th weekend	1.71 $\pm$ 0.55	4.93 $\pm$ 0.72 ⁽¹⁾	1.98 $\pm$ 0.40 ⁽³⁾⁽⁵⁾	6.22 $\pm$ 0.71 ⁽¹⁾⁽³⁾	79.746	0.000

Note: (1) $P < 0.01$ Compared with CG group; (2) $P < 0.05$ Compared with CG group; (3) $P < 0.01$ Compared with FG group; (4) $P < 0.05$ Compared with FG group; (5) $P < 0.01$ Compared with SBF group; (6) $P < 0.05$ Compared with SBF group.

3 讨论

5-HT 作为一种中枢神经系统内单胺类神经递质, 参与多

种脑功能如情绪、疼痛、睡眠、体温、内分泌等的调节^[78]。近年来, 5-HT 成为研究运动性疲劳领域的热点之一, 诸多研究表明, 5-HT 是脑内抑制性神经递质, 其代谢变化与运动性中枢疲

劳有关^[9,10]。

肝与运动性疲劳的发生有密切的关系,运动可以不同程度地影响肝脏组织细胞的结构、功能及抗氧化能力。研究表明,力竭性运动训练可导致血清谷丙转氨酶、谷草转氨酶活性显著升高,对肝脏产生不良影响^[11,12]。现代医学表明,运动时,血液的重新分布、能源物质的消耗和代谢产物的堆积等,也都会从不同方面、不同程度的影响肝脏组织的结构和功能,导致运动性疲劳的发生。

无论是一次性力竭运动还是长时间的运动,中枢 5-HT 都是增加的^[13],5-羟色胺增高可抑制大脑皮质发放冲动的能力,最终引起运动能力的下降,促使疲劳的产生。脑内 5-HT 的增加与脑中 Trp 浓度有关^[14],色氨酸是合成 5-HT 的前体,色氨酸(Trp)在血液中有两种存在形式,80%~90%与血清白蛋白结合即结合型,10%-20%为游离型即 F-Trp,只有 F-Trp 可通过血脑屏障,当血中 F-Trp 增多时,则进入脑组织也随之增多。同时,血清中性氨基酸尤其是 BCAA 可与 F-Trp 竞争这些载体,长时间运动由于能量需求增多,BCAA 分解增多,则 F-Trp 透过血脑屏障增加,脑中 F-Trp 增多,5-HT 合成增加。本研究发现:力竭运动导致大鼠血清 F-Trp、BCAA 含量的升高和下降,而经皮脉冲电流刺激疲劳大鼠肝区可有效缓解血清 F-Trp、BCAA 含量的升高和下降,疲劳前和疲劳后刺激无统计学意义。

运动不但使血清 F-Trp 含量增加,血清 BCAA 含量下降,而且还导致 F-Trp/BCAA 比值升高^[15],比值升高色氨酸进入大脑增加,5-HT 的生成加快含量升高,引起中枢疲劳的产生,其原因有以下两方面:一是运动中骨骼肌收缩,血中 BCAA 被氧化吸收供能,血清 BCAA 含量相应减少;二是运动使血浆中脂肪酸(FA)浓度增加,将白蛋白结合位点上松弛结合的 TRP 替换下来,从而导致血清中 F-TRP 相应升高^[16]。前期大量研究^[17,18]发现 BCAA 具有延缓疲劳发生、促进疲劳恢复的作用。本研究发现:力竭运动导致大鼠血清 F-Trp/BCAA 含量升高,而经皮脉冲电流刺激疲劳大鼠肝区可缓解血清 F-Trp/BCAA 含量的升高,可能与以下有关,其一经皮脉冲电流刺激疲劳大鼠肝区,加速了血液中 Trp 在肝脏的降解^[19];其二经皮脉冲电流刺激疲劳大鼠肝区,提高机体乳酸的清除,加速肝糖原的生成,BCAA 氧化供能相对减少,血中 BCAA 含量升高,从而导致 F-Trp/BCAA 含量的下降,说明经皮脉冲电流刺激肝区具有抗疲劳的作用。

力竭运动使血中 F-TRP / BCAA 比值升高,进而穿透血脑屏障的 F-TRP 增多,且色氨酸羟化酶(TPH)对其底物是不饱和的^[20],故中枢神经细胞合成的 5-HT 生成增多,5-HT 经单胺氧化酶(MAO)催化生成 5-羟吲哚乙醛及最终产物 5-HIAA。Bailey^[21]等研究发现大鼠跑台上运动 1 h、3h(力竭)后均使大鼠中脑、纹状体及下丘脑的 5-HT 和 5-HIAA 显著升高。本研究发现:FG 组大鼠纹状体 F-Trp、5-HT、5-HIAA 含量较 CG 组显著升高。第 5 周末:FG 组大鼠纹状体 5-HT 含量较第 3 周有下降趋势,但 5-HIAA 呈上升趋势,可能为机体产生了运动适应,与刘蓓蓓^[22]等研究结果一致;SAF 组大鼠纹状体 5-HT、5-HIAA 含量较 FG 组明显下降,可能与脉冲电流刺激疲劳大鼠肝区,肝脏供血增加,加速 TPH 和 MAO 代谢,5-HT 合成和降解相对减少有关;SBF 组大鼠纹状体 5-HT、5-HIAA 含量较 SAF 组明显升高,可能为疲劳前刺激加速了机体的 5-HT 合成和降解,导致了 5-HT、5-HIAA 的同步升高。

综上所述,疲劳后经皮脉冲电流刺激大鼠肝区可有效降低血清 F-Trp/BCAA、纹状体 5-HT 的含量,增强大鼠中枢神经系统的兴奋性,使其疲劳抑制状态得到缓解,具有抗中枢疲劳的作用。

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