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房颤大鼠模型心室重构与心肌细胞钙稳态和心律失常的关联 *

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摘要 目的:探讨房颤大鼠模型心室重构与心肌细胞钙稳态和心律失常的关联性。**方法:**将雄性 Wistar 大鼠随机平分为两组,各组 8 只,模型组采用乙酰胆碱 - 氯化钙混合液尾静脉注射法建立房颤动物模型,对照组注射同剂量的生理盐水,记录两组心室重构、心肌细胞钙稳态、心律失常情况并进行相关性分析。**结果:**模型组建模第 2 周与第 4 周的左室舒张末期内径(LVEDD)、左室收缩末期内径(LVESD)值都高于对照组(P<0.05)。模型组建模第 2 周与第 4 周的血清肌钙蛋白(cTnT)含量高于对照组(P<0.05)。模型组建模第 2 周与第 4 周心脏体外牵张性心律失常持续时间都高于对照组(P<0.05)。Pearson 相关分析显示建模第 2 周与第 4 周的 LVEDD、LVESD、cTnT 与牵张性心律失常持续时间存在正相关(P<0.05)。**结论:**房颤大鼠伴随有心室重构与心肌细胞钙离子的大量释放,可增加牵张性心律失常持续时间,相关性分析结果表明:心室重构、心肌细胞钙稳态和心律失常存在显著正相关性。

关键词:房颤;大鼠;心室重构;钙稳态;心律失常

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Association of Ventricular Remodeling, Calcium Homeostasis and Arrhythmia in Atrial Fibrillation Rat Model*

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ABSTRACT Objective: To investigate the relationship between ventricular remodeling and cardiac muscle cell calcium homeostasis and arrhythmia in atrial fibrillation rat models. **Methods:** Male Wistar rats were randomly divided into two groups, each group was eight. The model group were injected into the tail vein of acetylcholine-calcium chloride to establish atrial fibrillation animal model. The control group were injected with the same dose of normal saline, and the ventricular remodeling, myocardial cell calcium homeostasis, arrhythmia of the two groups were recorded and given correlation analysis. **Results:** The left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) values in the 2nd and 4th weeks of modeling in the model group were higher than those of the control group (P<0.05). The level of serum troponin (cTnT) in the model group were higher in the 2nd and 4thweeks of modeling than in the control group (P<0.05). The duration of the cardiac stretch arrhythmia in the 2nd and 4th weeks of modeling in the model group was higher than that in the control group (P<0.05). Pearson correlation analysis showed that LVEDD, LVESD, cTnT were positive correlated with the duration of stretch arrhythmia in the 2nd and 4th week of modeling (P<0.05). **Conclusion:** Atrial fibrillation rats are accompanied by ventricular remodeling and the release of large amounts of calcium ions in cardiomyocytes, which can increase the duration of tractive arrhythmia. Correlation analysis results show that: ventricular remodeling, cardiac muscle cell calcium homeostasis and arrhythmia have a significant positive correlation.

Key words: Atrial fibrillation; Rat; Ventricular remodeling; Calcium homeostasis; Arrhythmia

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前言

心房颤动简称房颤,是一种临床上比较常见的心律失常,男性的发病率高于女性,多发病于中老年人[1]。研究显示:心外膜脂肪组织扩张和促炎脂肪细胞因子的释放,导致微循环功能障碍和相邻心肌纤维化,导致心房颤动,另外,左心房的炎症变化导致电解剖重构[2,3]。房颤可导致机体出现容积和压力超负荷情况,激活了心肌细胞膜上牵张激活的离子通道,从而引起电生理特性的改变,诱发牵张性心律失常的发生[4-6]。心律失常可导致机体心排血量减少,使心率增快、心肌收缩力加强,激活交感神经系统,使又会增加心肌耗氧量、心肌收缩力减弱,造成肾素 - 血管紧张素系统激活,使心肌负荷过重,并促进交感神经

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兴奋,增加去甲肾上腺素的释放,从而形成恶性循环[7,8]。细胞外基质与心肌细胞的变化，属于房颤机体心室重构的结构基础，前者的变化主要是间质纤维化，后者主要包括了心肌细胞丧失[9,10]。房颤患者血清中肌钙蛋白(cTnT)含量高于正常人,cTnT心肌细胞的结构蛋白,可参与心肌细胞的电生理特性和机械特性的改变。房颤可导致心肌损伤,从而诱发 cTnT 的大量释放[11,12]。本文具体探讨了房颤大鼠模型心室重构与心肌细胞钙稳态和心律失常的关联,以期明确房颤发生的机制。

1 材料与方法

1.1 实验动物

36 只雄性 Wistar 大鼠购自北京市华阜康生物科技股份有限公司(合格证号 2020A014),体重(230±25)g。所有大鼠在实验动物中心进行饲养,大鼠可自由进食、饮水。光照时长按昼夜时长 12 h:12 h 的比例设定，饲养室温度控制在 20℃~22℃之间。cTnT 酶联免疫检测试剂盒购自上海生工公司,大鼠超声设备购自美国 GE 公司。

1.2 动物分组与处理

通过随机的方式对大鼠进行分为模型组与对照组各 8 只。① 模型组:采用乙酞胆碱 - 氯化钙混合液尾静脉注射法建立房颤动物模型,用 3 %戊巴比妥 30 mg/kg/ 只麻醉大鼠,肢体反射消失后四肢内侧备皮。经尾静脉给药,由尾静脉注入乙酞胆碱 - 氯化钙混合液(乙酞胆碱 66 μg/mL、氯化钙(10 mg/mL)1 mL/kg,于 5 s 内注完,1 次 /d,连续给药 7 d 后,心电图有典型房颤改变为房颤成功模型。② 对照组:经尾静脉注射与乙酞胆碱 - 氯化钙混合液等体积的生理盐水。

1.3 观察指标

1.3.1 心室重构指标检测 在建模第 2 周与 4 周采用超声测定左室舒张末期内径（left ventricular end-diastolic diameter, LVEDD)、左室收缩末期内径(left ventricular end-systolic diameter, LVESD)等反映心室重构的指标。

1.3.2 血清 cTnT 含量检测 在建模第 2 周与 4 周于腹主动脉取大概 1 mL 的血,静置于 37 ℃的水浴,时长为 0.5 h,取上层血清,采用双抗体夹心酶联免疫吸附测定法(enzyme linked immunosorbent assay, ELISA)检测 cTnT 含量。

1.3.3 心脏体外牵张性心律失常持续时间测量 在建模第 2 周与 4 周剪断大鼠肺静脉,将心脏游离剪下,移至恒温灌流装置中,经心尖部插入左室导管,通过注射器向乳胶球囊注 37℃温水,使左心室舒张末压增加 1.2 kPa 左右,记录瞬时膨胀 30 s 后心电图并测量两组大鼠心脏体外心律失常持续时间(persistent duration of arrhythmia,PDA)。

1.4 统计方法

选择 SPSS19.00 对本数据进行分析，均数±标准差表示计量数据，采用 t 检验进行组间对比;Pearson 相关分析进行相关性分析,检验水准为 α=0.05。

2 结果

2.1 心室重构指标对比

模型组建模第 2 周与第 4 周的 LVEDD、LVESD 值都高于对照组(P<0.05)。见表 1。

表 1 两组建模不同时间点的心室重构指标对比(cm)

Table 1 Comparison of ventricular reconstruction indexes between the two groups modeling at different time points (cm)

Groups	n	Modeling week 2		Modeling week 4	
		LVEDD	LVESD	LVEDD	LVESD
Control group	8	0.57±0.09	0.29±0.03	0.58±0.10	0.30±0.04
Model group	8	0.64±0.11	0.58±0.07	0.74±0.15	0.59±0.08
t		2.090	16.155	3.765	13.756
P		0.044	<0.001	0.001	<0.001

2.2 血清 cTnT 含量对比

模型组建模第 2 周与第 4 周的血清 cTnT 含量高于对照组(P<0.05)。见表 2。

表 2 两组建模不同时间点的血清 cTnT 含量对比(ng/L)

Table 2 Comparison of serum cTnT content between the two groups modeling at different time points (ng/L)

Groups	n	Modeling week 2	Modeling week 4
Control group	8	42.87±8.82	43.34±5.72
Model group	8	76.22±10.03	112.74±15.29
t		10.594	18.036
P		<0.001	<0.001

2.3 心脏体外牵张性心律失常持续时间对比

模型组建模第 2 周与第 4 周的牵张性心律失常持续时间都高于对照组(P<0.05)。见表 3。

2.4 相关性分析

在模型组中,Pearson 相关分析显示建模 2 周与第 4 周的 LVEDD、LVESD、cTnT 与牵张性心律失常持续时间存在显著正相关性(P<0.05)。见表 4。

表 3 两组不同时间点的心脏体外牵张性心律失常持续时间对比(s)

Table 3 Comparison of the duration of external cardiac stretch arrhythmia between the two groups at different time points(s)

Groups	n	Modeling week 2	Modeling week 4
Control group	8	0.92±0.10	0.98±0.08
Model group	8	2.33±0.41	1.36±0.11
t		9.450	11.853
P		<0.001	<0.001

表 4 心室重构与心肌细胞钙稳态和心律失常的关联

Table 4 Association of ventricular remodeling and cardiomyocyte calcium homeostasis and arrhythmias

Index		LVEDD	LVESD	cTnT
Modeling week 2	*r*	0.566	0.622	0.700
	P	0.001	0.000	0.000
Modeling week 4	*r*	0.422	0.455	0.633
	P	0.014	0.010	0.000

3 讨论

房颤是房性心律失常中最为常见的一种，可引起脑梗塞、心力衰竭等多种并发症，从而增加心脑血管疾病发病率和死亡率[13]。房颤的特点是电重构和结构重构，房颤期间不规则和 / 或快速房室传导可导致心肌动过速、压力和容量超负荷以及随后的扩张、瓣膜反流和心室功能障碍，并进展为心力衰竭[14,15]。房颤的确切发生机制还不明确，因此建立房颤动物模型，研究相关因素在房颤发生中的变化及作用具有重要价值。已有研究显示房颤发生时心房的有效收缩消失会导致心脏的排血量减少，从而引起心肌缺血损伤的发生[16,17]。本实验采用乙酞胆碱 - 氯化钙混合液尾静脉注射给药造成的房颤动物模型，乙酞胆碱与氯化钙可相互作用与促进，使心肌细胞膜钙通道短时间失活，增加心房肌细胞内钙负荷，减弱对异位兴奋的抑制，从而导致房颤的持续。本研究显示模型组建模第 2 周与第 4 周的 LVEDD、LVESD 值都高于对照组（*P*<0.05）。当前也有研究表明房颤大鼠可表现为右心室质量与室间隔质量比值显著升高，管腔显著狭窄，中膜平滑肌细胞增生伴玻璃样变性，可导致心房肌细胞电重构与组织重构，使得心室扩大，与本研究上述相关结果一致，并为上述 LVEDD、LVESD 的异常变化做出解释[18-20]。

房颤机体伴随有心房高频率起搏，引发心肌细胞内大量钙离子内流[21,22]。cTnT 是肌肉收缩的调节蛋白，具有心肌特异性。当心肌受到损伤时，cTnT 便可大量表达，为此可成为判断心肌缺血损伤的重要指标[23,24]。本研究显示模型组建模第 2 周与第 4 周的血清 cTnT 含量高于对照组（*P*<0.05）。当前有研究[25,26]表明：血清 cTnT 是一项与房颤相关的重要、独立的因素，绝大多数正常人 cTnI<0.4 ng/mL，而多数房颤患者 cTnI>0.4 ng/mL，另外，并且心房快速激动引起频率依赖性钙离子通道激活，导致钙超载，心房通过自身适应性调节，降低心房收缩功能，使得心房扩大，从而诱发房颤的形成，与本研究结果一致。

本研究显示模型组建模第 2 周与第 4 周的牵张性心律失常持续时间都高于对照组（*P*<0.05），结合 Yang M[27]和苏哲[28]等相关研究分析：房颤可膨胀左心室，舒张末压相应增加使单个心室肌细胞受到机械牵张，引起牵张性心律失常。相关研究显示：牵张性心律失常也是房颤向心力衰竭发展病理生理进程中的关键环节，抑制和逆转牵张性心律失常的发生一直是房颤防治的重点[29,30]。另外，房颤机体的心室重构与钙离子通道的变化是房颤细胞电生理的主要表现，两个方面的相互作用构成了房颤发生和持续的病理生理基础[31,32]。本研究 Pearson 相关分析显示模型组建模第 2 周与第 4 周的 LVEDD、LVESD、cTnT 与牵张性心律失常持续时间存在相关性（*P*<0.05）。本研究也存在一定的不足，没有进行细胞学分析，取样的时间点比较少，且大鼠样本数量较少，将在后续研究中进行深入分析。

综上所述，房颤大鼠伴随有心室重构与心肌细胞钙离子的大量释放，可增加牵张性心律失常持续时间，相关性分析结果表明：心室重构、心肌细胞钙稳态和心律失常存在显著正相关性。

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