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# 原发性醛固酮增多症大鼠自主活动和学习记忆行为的影响 *

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**摘要 目的：**探讨原发性醛固酮增多症(primary aldosteronism，PA)大鼠其自主活动和对学习记忆行为的影响。**方法：**8 周龄健康雄性 SD(Sprague-Dawley)大鼠(n=30)随机分为对照组与模型组各 15 只。两组都皮下埋置微量渗透泵，模型组泵内灌注醛固酮，对照组泵内灌注等量的生理盐水，记录大鼠自主活动和学习记忆行为的变化情况。**结果：**所有大鼠均存活，模型组都造模成功，切口愈合良好。模型组造模后的收缩压高于对照组($P$<0.05)，也高于造模前($P$<0.05)，两组造模前后心率对比差异无统计学意义($P$>0.05)。模型组造模后的逃避潜伏期与穿台次数少于对照组($P$<0.05)，也少于造模前($P$<0.05)。模型组造模后的自主活动次数高于对照组($P$<0.05)，也高于造模前($P$<0.05)。造模后模型组的鼠双微基因 2(Mouse Double Microgene 2，MDM2)蛋白相对表达水平高于对照组($P$<0.05)。造模后模型组的血清醛固酮含量都高于对照组($P$<0.05)，血清钾离子、钠离子、肾素活性低于对照组($P$<0.05)。**结论：**原发性醛固酮增多症大鼠伴随有血清钾离子、钠离子含量降低与 MDM2 蛋白的高表达，从而导致大鼠出现自主活动和学习记忆行为障碍。

**关键词：**原发性醛固酮增多症；大鼠；自主活动；学习记忆行为；鼠双微基因 2
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# Effects of Autonomous Activities, Learning and Memory Behaviors in Rats with Primary Aldosteronism*

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**ABSTRACT Objective:** To investigate the effects of primary aldosteronism (primary aldosteronism, PA) rats on autonomous activities and learning and memory behavior. **Methods:** 8-week-old healthy male SD (Sprague-Dawley) rats (n=30) were randomly divided into control group and model group with 15 rats in each groups. Micro-osmotic pumps were implanted subcutaneously in both groups. The model group were perfused with aldosterone, and the control group were perfused with the same amount of normal saline. The changes in autonomous activities and learning and memory behavior of rats were recorded. **Results:** All rats were alive, the model group were successfully, and the incisions healed well. The systolic blood pressure of the model group after modeling were higher than that of the control group ($P$<0.05), and were also higher than before modeling($P$<0.05). There were no statistically significant difference in heart rate compared between the two groups before and after modeling ($P$>0.05). The escape latency and the number of crossings after modeling in the model group were less than those in the control group ($P$<0.05), and were also less than before ($P$<0.05). The number of autonomous activities in the model group after modeling were higher than that in the control group ($P$<0.05), and were also higher than before modeling ($P$<0.05). After modeling, the relative expression level of Mouse Double Microgene 2 (MDM2) protein in the model group were higher than that in the control group ($P$<0.05). After modeling, the serum aldosterone content of the model group were higher than that of the control group ($P$<0.05), and the serum potassium ion, sodium ion and renin activity were lower than those of the control group ($P$<0.05). **Conclusion:** Primary aldosteronism rats are accompanied by decreased serum potassium and sodium levels and high expression of MDM2 protein, which lead to disorders of autonomous activities and learning and memory behavior in rats.

**Key words:** Primary aldosteronism; Rats; Autonomous activities; Learning and memory behavior; Mouse double microgene 2
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## 前言

原发性醛固酮增多症(primary aldosteronism,PA)是继发性高血压的常见原因之一,是因肾上腺分泌过多醛固酮所引起的的临床疾病[1,2]。临床上认为原发性醛固酮增多症导致盐皮质激素分泌异常相关继发性高血压发生的主要原因[3]。随着医学诊疗技术的提高,原发性醛固酮增多症导致的高血压已占总高血压人群的 8 %左右[4,5]。醛固酮的作用是促进肾脏集合系统对钠离子和水的重吸收,从而维持人体体液代谢的平衡[6]。醛固酮的大量释放可引起心脏肥大、血管重构等病变,也可对其他多个脏器器官造成损伤。特别是醛固酮通过与其受体结合后,能引起肾脏的炎症性损伤及纤维化的形成, 从而引起大脑损伤,影响机体的认知功能[7,8]。建立动物模型是研究原发性醛固酮增多症发病机制的重要手段。目前在体内实验中很难维持肾上腺肿瘤细胞的内分泌特性,很难真实模拟醛固酮腺瘤持续分泌激素的过程[9,10]。为此当前多采用通过在动物体内皮下埋置微量渗透泵,利用大鼠等渗与渗透泵内高渗液之间的渗透压差产生的驱动作用,持续释放醛固酮溶液,从而可模拟建立原发性醛固酮增多症动物模型[11,12]。本文具体探讨了原发性醛固酮增多症大鼠自主活动和学习记忆行为的影响,以明确原发性醛固酮增多症的作用机制。现总结报道如下。

## 1 材料与方法

### 1.1 动物与试剂

8 w 龄健康雄性 SD(Sprague-Dawley)大鼠(n=30)购自上海赛默科技生物发展有限公司,体质量 220~240 g,清洁级。饲养于本医院动物实验中心提供,本院动物伦理委员会批准了此次研究。饲养条件:室温 22℃~25℃,自由饮水饮食,光线 12 h 明暗交替。醛固酮购于美国 Sigma-Aldrich 公司,放免试剂盒[醛固酮与肾素活性]购自北京北方生物技术研究所,微量渗透泵购自美国 Alzet,Durect 公司,Morri 水迷宫行为试验系统购自北京金盛恒星医疗器械公司,自主活动仪购自安徽淮北正华生物仪器有限公司。

### 1.2 动物分组与处理

14 根据随机数字表法把大鼠分为对照组与模型组各 15 只。模型组:泵内灌注醛固酮,释放速度 1 g/h。20 %乌拉坦腹腔麻醉,常规消毒,将大鼠肩胛区背部皮肤用 8 %硫化钠进行脱毛,沿肩胛区作横行皮肤切口,用长弯血管钳向尾侧纵向分离皮下间隙。将微量渗透泵埋植在大鼠背部肩胛区皮下间隙内,持续作用 4 w。对照组:微量渗透泵泵出的为等量的生理盐水。

### 1.3 观察指标

(1) 在造模前与造模后在大鼠安静状态下用尾套法测量心率、收缩压,测定 3 次取平均值。(2)在造模后麻醉大鼠后,显露并游离下腔静脉,抽取静脉血,检测血清钾离子、钠离子、醛固酮与肾素活性。(3)在造模前与造模后进行 Morris 水迷宫与自主活动实验,其中 Morris 水迷宫实验包括定位航行实验和空间探索实验,记录大鼠逃避潜伏期与穿过原平台的次数。在自主活动实验,将鼠同时分入自主活动仪的盒内,活动仪自动记录大鼠 5 min 内的活动次数。(4)造模后处死大鼠,完全暴露胸、腹腔,切取全长主动脉,研磨后采用细胞裂解液提取组织总蛋白, 采用 Western blot 法检测鼠双微基因 2 (Mouse Double Microgene 2,MDM2)蛋白相对表达水平,以 MDM2/β-actin 灰度比值作为 MDM2 蛋白的相对表达量。

### 1.4 统计方法

采用 SPSS 23.00 统计软件分析进行分析,数据以均数±标准差表示, 组间比较应用 LSD 检验, 检验水准为 $\alpha$=0.05,以 $P$<0.05 为差异有统计学意义。

## 2 结果

### 2.1 两组心率、收缩压对比

所有大鼠均存活,模型组都造模成功,切口愈合良好。模型组造模后的收缩压高于对照组($P$<0.05),也高于造模前($P$<0.05),两组造模前后心率对比差异无统计学意义($P$>0.05),见表 1。

表 1 两组造模前后心率与收缩压对比($\bar{x}\pm s$)

Table 1 Comparison of heart rate and systolic blood pressure between the two groups before and after modeling ($\bar{x}\pm s$)

| Groups | n | Heart rate (min) | | SBP (mmHg) | |
|---|---|---|---|---|---|
| | | Before molding | After molding | Before molding | After molding |
| Model group | 15 | 313.22±24.18 | 312.55±18.47 | 100.34±8.19 | 138.66±14.02*# |
| Control group | 15 | 312.87±18.39 | 313.29±20.74 | 101.76±10.47 | 100.63±9.14 |

Note: Compared with the control group, *$P$<0.05; Compared with before molding, #$P$<0.05.

### 2.2 两组学习记忆行为对比

模型组造模后的逃避潜伏期与穿台次数少于对照组($P$<0.05),也少于造模前,对比有统计学意义($P$<0.05),见表 2。

### 2.3 两组自主活动对比

模型组造模后的自主活动次数高于对照组(P<0.05),也高于造模前,对比有统计学意义($P$<0.05),见表 3。

### 2.4 两组 MDM2 蛋白相对表达水平对比

造模后模型组的 MDM2 蛋白相对表达水平高于对照组,对比有统计学意义($P$<0.05),见表 4。

### 2.5 两组血清钾离子、钠离子、醛固酮与肾素活性对比

造模后模型组的血清醛固酮含量都高于对照组($P$<0.05),血清钾离子、钠离子、肾素活性低于对照组,对比有统计学意义($P$<0.05),见表 5。

## 3 讨论

原发性醛固酮增多症导致的继发性高血压逐年增加,其中以分泌醛固酮的肾上腺皮质腺瘤最为常见。该病在临床上除了表现为高血压外,还可表现为低肾素、碱中毒、高醛固酮、低血

表 2 两组造模前后学习记忆行为能力对比($\bar{x}\pm s$)

Table 2 Comparison of learning and memory behavior ability between the two groups before and after modeling ($\bar{x}\pm s$)

| Groups | n | Escaping incubation period (s) | | Number of passes (times) | |
|---|---|---|---|---|---|
| | | Before molding | After molding | Before molding | After molding |
| Model group | 15 | 33.56±4.19 | 26.09±2.78*# | 4.56±0.45 | 3.13±0.47*# |
| Control group | 15 | 33.10±4.10 | 33.76±3.17 | 4.51±0.33 | 4.53±0.72 |

Note: Compared with the control group, *$P$<0.05; Compared with before molding, #$P$<0.05.

表 3 两组造模前后自主活动次数对比(次，$\bar{x}\pm s$)

Table 3 Comparison of the number of autonomous activities between the two groups before and after model building (times, $\bar{x}\pm s$)

| Groups | n | Before molding | After molding |
|---|---|---|---|
| Model group | 15 | 108.22±21.47 | 28.14±3.19*# |
| Control group | 15 | 109.76±17.22 | 111.76±21.74 |

Note: Compared with the control group, *$P$<0.05; Compared with before molding, #$P$<0.05.

表 4 两组造模后 MDM2 蛋白相对表达水平对比($\bar{x}\pm s$)

Table 4 Comparison of the relative expression levels of MDM2 protein between the two groups after modeling ($\bar{x}\pm s$)

| Groups | n | Relative expression of MDM2 protein |
|---|---|---|
| Model group | 15 | 0.98±0.11* |
| Control group | 15 | 0.39±0.08 |

Note: Compared with the control group, *$P$<0.05.

表 5 两组血清钾离子、钠离子、醛固酮与肾素活性对比($\bar{x}\pm s$)

Table 5 Comparison of serum potassium ion, sodium ion, aldosterone and renin activity between the two groups ($\bar{x}\pm s$)

| Groups | n | Potassium ion (mmol/L) | Sodium ion (mmol/L) | Aldosterone (ng/L) | Renin activity (μg/L· $h^{-1}$) |
|---|---|---|---|---|---|
| Model group | 15 | 3.33±0.21* | 135.39±11.47* | 308.27±24.51* | 3.82±0.22* |
| Control group | 15 | 5.62±0.76 | 144.08±12.47 | 80.17±11.33 | 18.47±2.22 |

Note: Compared with the control group, *$P$<0.05.

钾、肌无力、夜尿增多等症状[13,14]。特别是醛固酮可以不依赖血流动力学变化，通过参与钙化、氧化应激、纤维化、细胞周期的调控，从而对机体其他组织器官造成损伤[15]。不过当前对于醛固酮致病作用机制的研究多为体外实验研究，很难模拟人体内环境因素的影响。本研究显示所有大鼠均存活，模型组都造模成功，切口愈合良好；模型组造模后的收缩压高于对照组，也高于造模前，两组造模前后心率对比差异无统计学意义；造模后模型组的血清醛固酮含量都高于对照组，血清钾离子、钠离子、肾素活性低于对照组，说明本研究建立的动物模型与临床上的 Funder JW[16]调查的原发性醛固酮增多症患者特点相类似。从机制上分析，在本次研究建立的原发性醛固酮增多症大鼠模型中，微量渗透泵由两层膜、两个腔组成，内、外腔之间由非通透性膜隔开[17]。外腔为密闭结构，其内充以高渗盐水；内腔为药物舱，用来灌注所研究的药物。可利用渗透泵渗透压差产生的驱动作用，持续释放泵内的醛固酮，可在体内模拟产醛固酮的肾上腺腺瘤作用过程[18,19]。不过在具体的建模过程中要注意以下事项：(1)1g/h 的渗透泵醛固酮的释放量，可抑制血浆肾素活性及血管紧张素 II 的浓度[20]；(2)渗透泵安放前可事先浸没在无菌生理盐水中，于 37℃条件下放置 36~48 h，有利于醛固酮液达到恒速排放状态；(3)以丙二醇：无水乙醇：双蒸水为 6：1：3 体积比配制溶解醛固酮的效果最好[21]。

醛固酮受体除了存在于肾脏集合管外，还存在机体的大脑组织中。本研究显示模型组造模后的逃避潜伏期与穿台次数少于对照组，也少于造模前；模型组造模后的自主活动次数高于对照组，也高于造模前，与 Vassiliadi DA[22]、Wannachalee T[23]等学者的研究结果类似，也表明原发性醛固酮增多症大鼠多伴随有自主活动和学习记忆行为障碍。表明原发性醛固酮增多症大鼠多伴随有自主活动和学习记忆行为障碍。自主活动和学习记忆行为标志着动物中枢神经系统的兴奋状态，两者发生障碍反应了原发性醛固酮增多症大鼠的精神行为障碍，与临床患者表现一定的焦虑抑郁情绪相一致[24,25]。然后各种神经递质在不同区域脑内的变化错综复杂，原发性醛固酮增多症对大鼠精神行为的影响还有待继续分析。

本研究显示造模后模型组的 MDM2 蛋白相对表达水平高于对照组，表明醛固酮可能能促进血管平滑肌细胞 MDM2 的表达，与 Reincke M[26]、Shea SA[27]等学者的研究结果类似。从机制上分析，鼠双微基因 2(murine double mimute 2，MDM2)序列在进化上高度保守，可参与调节细胞的生长、凋亡过程，MDM 过度表达可促进细胞的增殖[28-30]。醛固酮可刺激血管平滑肌细胞的增殖，诱发机体细胞外基质、血管壁细胞、细胞因子和黏附

分子的表达发生变化,可导致 MDM2 蛋白表达异常。本研究也存在一定的不足,没有设置抑制组,没有进行相关性分析,将在后续研究中进行探讨。

总之,原发性醛固酮增多症大鼠伴随有血清钾离子、钠离子含量降低与 MDM2 蛋白的高表达,从而导致大鼠出现自主活动和学习记忆行为障碍。

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