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卡托普利对肾血管性高血压大鼠血小板胞浆$[Ca^{2+}]_i$及血浆TXA_2/PGI_2的影响

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Effect of captopril on platelet cytosolic $[Ca^{2+}]_i$ and plasma TXA_2/PGI_2 in renovascular hypertensive rats

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KEY WORDS blood pressure; renovascular hypertension; captopril; calcium; synthetic prostaglandins F; epoprostenol; thromboxane A_2; platelet aggregation; angiotensin Ⅱ

AIM: To study the effect of captopril (Cap) on platelet cytosolic free calcium concentration ($[Ca^{2+}]_i$), platelet aggregation (PAg), and plasma TXA_2/PGI_2 ratio in the renovascular hypertensive rats. **METHODS**: Blood pressure was measured once a week by tail-cuff microphonic manometer. Platelet $[Ca^{2+}]_i$ was measured by Fura 2-AM. Plasma angiotensin Ⅱ (Ang), thromboxane A_2 (TXA_2), and prostacycline (PGI_2) were measured by radioimmunoassay. **RESULTS**: Platelet $[Ca^{2+}]_i$ and PAg increased ($P<0.01$), while plasma Ang and TXA_2/PGI_2 ratio elevated ($P<0.05$) in the renovascular hypertensive rats; platelet $[Ca^{2+}]_i$ and plasma TXA_2/PGI_2 ratio reduced markedly after ig Cap 100 mg·kg^{-1}·d^{-1} compared with saline for 2 wk. **CONCLUSION**: The altered TXA_2/PGI_2 after Cap treatment contributed to the improvement of the platelet $[Ca^{2+}]_i$ and PAg.

关键词 血压；肾血管高血压；卡托普利；钙；合成前列腺素 F 类；前列腺环素醇；血栓素 A_2；血小板聚集；血管紧张素Ⅱ

目的：观察卡托普利（Cap）对血浆血栓素 A_2（TXA_2）、前列腺环素（PGI_2）及血小板胞浆钙离子浓度（$[Ca^{2+}]_i$）、血小板聚集率（PAg）的影响. **方法**：大鼠管饲 Cap 100 mg·kg^{-1}·d^{-1}，2 wk. **结果**：二肾一夹型肾血管性高血压大鼠血浆血管紧张素Ⅱ（Ang）和 TXA_2/PGI_2 升高（$P<0.05$），血小板 $[Ca^{2+}]_i$ 及 PAg 显著增高（$P<0.01$）. Cap 在显著降低血压同时，血浆 TXA_2/PGI_2 显著降低（$P<0.05$），且血小板 $[Ca^{2+}]_i$ 及 PAg 也明显降低（$P<0.01$）. **结论**：Cap 对血小板 $[Ca^{2+}]_i$ 及血小板功能的影响与其改善 TXA_2/PGI_2 比值有关.

血管紧张素Ⅰ转换酶抑制剂卡托普利（Cap）降血压作用不仅涉及肾素-血管紧张素系统，还有缓激肽、前列腺素等的参与. 缓激肽诱导内皮细胞生成一氧化氮和前列腺环素（PGI_2），升高细胞内环磷酸腺苷（cAMP），使血管舒张[1]. 血小板血栓素 A_2（TXA_2）的生成与血小板 $[Ca^{2+}]_i$ 存在有相互促进作用，血小板胞膜及核膜也具有血管紧张素Ⅱ（Ang）受体，Ang 可快速地升高血小板 $[Ca^{2+}]_i$[2]. Cap 在降压过程中降低血小板聚集与减少血小板 TXA_2 生成相平行[3]. Cap 对血小板 $[Ca^{2+}]_i$ 及血浆 TXA_2/PGI_2 的作用未见报道. 本文观察 Cap 对肾血管性高血压大鼠血浆 TXA_2/PGI_2 及血小板 $[Ca^{2+}]_i$、血小板聚集（PAg）的影响.

MATERIALS AND METHODS

模型建立[4] Wistar 大鼠 26 只（普通级，合格证号：94002，购自第一军医大学实验动物中心），鼠龄 12 wk. ♀♂各半，体重 150-200 g. 随机分组：手术组 20 只（分成 Hypertensive 组 6 只，Captopril 组 7 只，Saline 组 7 只），假手术组 6 只. 普通饲料喂养，自由饮水，光照 12 小时，无噪音. 大鼠戊巴比妥钠（40 g·kg^{-1}，ip）麻醉，右侧开腹，剥离右肾动脉主干并放置 0.25 mm 银夹（假手术组未置银夹，余同），缝合切口. 大鼠无创血压测量应用 CRS-Ⅲ型大鼠血压仪（浙江康乐仪器厂）监测大鼠在清醒状态下的鼠尾收缩压[5]. 每周一次. 实验第 10 周，由颈动脉取血，各血浆样品置 -20℃储存待测.

Cap 慢性降压实验[6] 术后第 6 周 Cap 组以每只 Cap

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100 mg·kg^{-1}(常州制药厂，白色粉剂，纯度98.5 %，生理盐水配制)管饲，每天 1 次，连续两周；对照组以每只生理盐水 5 mL·kg^{-1}管饲。观察血压变化，每周一次。

血浆 TXA_2/PGI_2 的测定 采用放免法检测血浆 TXA_2 及 PGI_2 代谢产物 TXB_2 和 6-keto-PGF_{1a}，用 TXB_2/6-keto-PGF_{1a}表示 TXA_2/PGI_2。药盒购自苏州医学院血栓与止血研究所。在γ计数仪(西安 262 厂制造)测定计数值。

血浆 Ang 的测定 放免法检测血浆 Ang 浓度，药盒购自北方同位素公司。在γ计数仪(西安 262 厂制造)测定计数值。

血小板聚集率的测定[7] 制备富血小板血浆及贫血小板血浆，加入二磷酸腺苷(ADP)作为诱导剂(终浓度 5 μmol·L^{-1})，应用 DAM-1 型聚集仪(江苏丹阳仪器厂制造)测定最大聚集率。

血小板内 $[Ca^{2+}]_i$ 的测定 采用双波长(340/380 nm)荧光法[8]，将钙指示剂 Fura 2-AM (Sigma)负载血小板内，依次加入 $CaCl_2$ 1 mmol·L^{-1}，Triton X-100 0.1 % 和 egtazic acid 3 mmol·L^{-1}，用 RF-5000 荧光分光光度计(日本 Nihon Konden 公司)分别测定基础、最大、最小的荧光比值。

所有数据由 $\bar{x} \pm s$ 表示，组间显著性比较用组间 t 检验；手术前后血压显著性比较用配对 t 检验。

RESULTS

收缩压变化 手术组大鼠在第 4 周末形成稳定高血压(22.1 ± 1.5 kPa *vs* 14.7 ± 1.9 kPa，n = 20，$P < 0.01$)；假手术组大鼠血压手术前后无明显变化(14.7 ± 1.3 kPa *vs* 14.1 ± 1.6 kPa，n = 6，$P > 0.05$)。

二肾一夹型肾血管性高血压大鼠与假手术大鼠相比，血浆 TXA_2/PGI_2 升高($P < 0.05$)，血小板内$[Ca^{2+}]_i$ 及 PAg 显著增高($P < 0.01$)，而血浆 Ang 无变化($P > 0.05$)(Tab 1)。

Tab 1. Levels of BP, angiotensin Ⅱ (Ang), TXA_2/PGI_2, platelet $[Ca^{2+}]_i$, and platelet aggregation (PAg) in hypertensive rats. $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* sham.

	Sham	Hypertensive
SBP/kPa	16.0 ± 1.5	22.0 ± 2.0[c]
Ang/ng·L^{-1}	400 ± 100	470 ± 107[a]
TXA_2/PGI_2	2.6 ± 0.6	4.2 ± 1.0[b]
$[Ca^{2+}]_i$/nmol·L^{-1}	92 ± 26	280 ± 47[c]
PAg/ %	29 ± 5	63 ± 13[c]

Cap 在显著降低血压同时，血浆 TXA_2/PGI_2 显著降低($P < 0.05$)，且血小板内$[Ca^{2+}]_i$ 及 PAg 也明显降低($P < 0.01$)。Cap 对血浆 Ang 无影响($P > 0.05$)(Tab 2)。

Tab 2. Effect of captopril on BP, angiotensin Ⅱ (Ang), TXA_2/PGI_2, platelet $[Ca^{2+}]_i$, and platelet aggregation (PAg) in hypertensive rats. $n = 7$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* saline.

	Saline	Captopril
SBP/kPa	21.9 ± 2.0	7.7 ± 1.3[c]
Ang/ng·L^{-1}	350 ± 113	310 ± 115[a]
TXA_2/PGI_2	4.1 ± 1.3	2.8 ± 1.4[b]
$[Ca^{2+}]_i$/nmol·L^{-1}	280 ± 33	170 ± 41[c]
PAg/ %	60 ± 10	36 ± 5[c]

DISCUSSION

本实验显示：二肾一夹型肾血管性高血压大鼠血浆 TXA_2/PGI_2 与假手术组相比显著升高($P < 0.05$)，血小板$[Ca^{2+}]_i$ 及其聚集率也明显增高($P < 0.01$)，表明肾血管性高血压大鼠存在血小板内 Ca^{2+} 及 TXA_2/PGI_2 代谢异常，与文献报道相似[9,10]。

Tab 2 显示：Cap 在显著降低血压同时，血浆 Ang 无明显变化，血浆 TXA_2/PGI_2 显著降低($P < 0.05$)，而且血小板$[Ca^{2+}]_i$ 及血小板聚集率也显著降低($P < 0.01$)。在二肾一夹型高血压大鼠早期(第 4 周)血浆和主动脉 Ang 水平升高，慢性期(第 8－12 周)血浆水平趋于正常，但主动脉 Ang 仍处于高水平[4]；而且 Cap 降压作用与局部组织肾素系统活性存在明显相关[6]。有报道 Cap 降低血小板聚集功能与减少血小板 TXA_2 的生成相平行[3]。本实验结果表明 Cap 对肾血管性高血压大鼠血小板$[Ca^{2+}]_i$ 及血小板聚集率的影响与其改善 TXA_2/PGI_2 比值有关。

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二氢埃托啡耐受后小鼠脑内 cAMP 含量的减少及氨基酸含量的增加[1]

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Decrease of cAMP and increase of amino acids contents in mouse brain after dihydroetorphine tolerance[1]

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KEY WORDS dihydroetorphine; drug tolerance; cyclic AMP; glutamic acid; aspartic acid; glutamine; GABA

AIM: To study the mechanism of dihydroetorphine (DHE) tolerance. **METHODS**: DHE tolerance was produced by repeated sc injections in progressively increased doses to mice for 8 d. The concentrations of amino acids and cAMP were detected by RP-HPLC/fluorescence assay and radioimmunoassay, respectively. **RESULTS**: The basal contents of glutamic acid (Glu), aspartic acid (Asp), and GABA in whole brain (cerebellum removed) were increased respectively from 14.1 ± 2.1, 3.0 ± 0.4, and 1.8 ± 0.8 μmol/g tissue in control mice to 17.2 ± 2.2, 4.1 ± 0.6, and 3.2 ± 1.0 μmol/g tissue in tolerant mice, and the rates of increase were 22.0 % ($P < 0.05$), 36.7 % ($P < 0.01$), and 77.8 % ($P < 0.05$ *vs* control), respectively. There was no significant difference in the basal contents of Gl_n (5.1 ± 1.0 *vs* 4.5 ± 1.7 μmol/g tissue of control). The basal contents of cAMP in hypothalamus and striatum were decreased respectively from 271 ± 38 and 189 ± 31 nmol/g tissue in control mice to 96 ± 15 and 65 ± 21 nmol/g tissue in tolerant mice ($P < 0.01$), and the rates of decrease were 64.6 % and 65.6 %, respectively. There was no significant difference of cAMP in cerebral cortex (72 ± 20 *vs* 55 ± 15 nmol/g tissue of control). **CONCLUSION**: The increases of Glu, Asp, and GABA in brain and the decrease of cAMP in hypothalamus and striatum were involved in DHE tolerance.

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关键词 二氢埃托啡; 药物耐受性; 环腺苷一磷