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BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 May; 15 (3): 279-281

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苦参碱对大鼠输精管的作用与激活钙通道的关系

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Relation of effects of matrine on rat *vasa deferens* to activation of calcium channels

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ABSTRACT Effective mechanisms of matrine (Mat) in contraction were observed in isolated rat *vasa deferens*. Mat caused a strong concentration-dependent contraction of *vasa deferens*, and this contraction was competitively inhibited by prazosin (Pra, 10 $\mu\text{mol}\cdot\text{L}^{-1}$) and

nifedipine (Nif, 50 $\text{nmol}\cdot\text{L}^{-1}$), with depression of maximal responses. Their pA_2 value was 5.1 and 9.29, respectively. The contraction was also inhibited by verapamil (Ver, 1 $\mu\text{mol}\cdot\text{L}^{-1}$) with depression of maximal responses; but this antagonism was noncompetitive. Its pD_2 value was 6.07. Mat promoted CaCl_2 -induced contraction of *vas deferens*. The effect of Mat was enhanced in proportion to increase in concentrations of CaCl_2 . Mat markedly strengthened KCl-induced contraction of *vas deferens*. The results suggest that one of the mechanisms of the contractive effects of Mat within a certain range of concentrations was related to the activation of the calcium channel.

Received 1992-08-10

Accepted 1993-10-12

(部分内容见下页)

KEY WORDS matrine; *ras deferens*; prazosin; nifedipine; verapamil; drug dose-response relationship; calcium channels

A 摘要 哌唑嗪 ($10 \mu\text{mol}\cdot\text{L}^{-1}$), 硝苯地平 ($50 \text{nmol}\cdot\text{L}^{-1}$) 可竞争性拮抗苦参碱收缩大鼠离体输精管, pA_2 值分别为 5.1 和 9.29. 维拉帕米 ($1 \mu\text{mol}\cdot\text{L}^{-1}$) 可非竞争性拮抗苦参碱, pD_2 为 6.07. 苦参碱可增强 CaCl_2 和 KCl 收缩输精管的作用, 对 KCl 的增强作用更显著. 提示苦参碱是一钙通道激动剂.

关键词 苦参碱; 输精管; 哌唑嗪; 硝苯地平; 维拉帕米; 药物剂量-效应关系; 钙通道

苦参碱(matrine, Mat)可从多种豆科槐属植物中提得. 本实验用 Mat 来源于苦豆子 (*Sophora alopecuroides* L.)^[1]. Mat 有加强豚鼠乳头状肌收缩的作用^[2]. 对豚鼠心房也有明显的正性肌力作用^[3]. Mat 的正性肌力作用与 Ca^{2+} 有关^[4]. 本文用大鼠离体输精管来观察 Mat 正性肌力作用与钙通道的关系.

MATERIALS

Mat (mp $75.5-77.5 \text{C}$), 为浅黄色结晶性粉末, 宁夏盐池制药厂产品. 用适量稀 HCl 溶解, 然后调 pH 至 7.5, 用生理溶液稀释至所需浓度. 哌唑嗪 (prazosin, Pra) 为北京制药工业公司产品. 普萘洛尔 (propranolol, Pro) 针剂为北京制药厂产品. 硝苯地平 (nifedipine, Nif) 和维拉帕米 (verapamil, Ver) 针剂均为天津医药工业研究所产品. 无水 CaCl_2 及 KCl 均为北京化工厂产品 AR 级.

METHODS AND RESULTS

Wistar 大鼠, ♂, 体重 $197 \pm 16 \text{g}$, 断头放血, 取双侧附睾端输精管约 15 mm. 一侧用作对照, 另一侧给阻断剂, 两侧交替实验 (因为 Mat 不易洗掉). 浴槽内 10 ml Krebs-Henseleit (K-H) 溶液, 温度 37C , 通 O_2 , pH 7.4,

负荷 1 g. 标本给予频率 1 Hz, 波宽 5 ms, 刺激强度 2 倍于阈刺激的电激动. 平衡 1 h 后开始实验. 其间每 15 min 换液一次, 肌条收缩通过张力换能器记录在 XWT-100 型台式自动平衡记录仪上. 分别制作药物的累加量-效曲线. 按文献^[5]计算 pA_2 或 pD_2 值.

Pra 对 Mat 收缩输精管作用的影响 实验前 15 min 加 Pro $1 \mu\text{mol}\cdot\text{L}^{-1}$, 以累积给药法得 Mat 增加输精管收缩作用的量-效曲线. Mat 的终浓度从 $0.2 \text{mmol}\cdot\text{L}^{-1}$ 开始, 以 3/4 递增至 $10.0 \text{mmol}\cdot\text{L}^{-1}$. 每次加入 Mat 总容量为 0.1 ml. 另一侧标本, 在给累加量 Mat 之前 15 min 给 Pra $10 \mu\text{mol}\cdot\text{L}^{-1}$. 结果 Pra 可竞争性拮抗 Mat 的收缩作用, 但最大反应降低. pA_2 值为 5.1 (Fig 1A).

Nif 和 Ver 对 Mat 收缩输精管作用的影响 Mat 的累加量-效曲线制作同上. 另一侧标本在给累加量 Mat 前 15 min 分别加入 Nif $50 \text{nmol}\cdot\text{L}^{-1}$, Ver $1 \mu\text{mol}\cdot\text{L}^{-1}$, 每个标本只给一个拮抗剂. 结果 Nif 亦可竞争性拮抗 Mat 的收缩作用, 但最大反应明显降低. 其 pA_2 值为 9.29. Ver 可非竞争性拮抗 Mat, 使收缩减弱, 最大反应压低. 其 pD_2 值为 6.07 (Fig 1B).

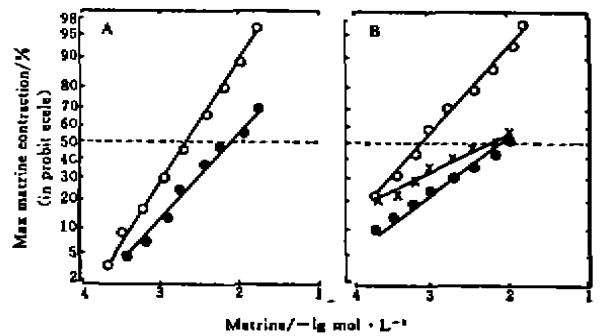


Fig 1. Cumulative dose-response curve of contractive effects of matrine on isolated rat *vas deferens*. A: (○) control (n=9), (●) prazosin $10 \mu\text{mol}\cdot\text{L}^{-1}$ (n=9); B: (○) control (n=17), (●) nifedipine $50 \text{nmol}\cdot\text{L}^{-1}$ (n=9), (×) verapamil $1 \mu\text{mol}\cdot\text{L}^{-1}$ (n=8).

Mat 对 CaCl₂ 收缩输精管作用的影响 将输精管标本置于无 Ca²⁺ K-H 液中平衡 1 h, 换无 Ca²⁺ 高 K⁺ (KCl 44.7 mmol·L⁻¹) 的极化液使肌条去极化, 15 min 后加累积浓度的 CaCl₂^[6]. 用无 Ca²⁺ K-H 液冲洗, 使标本回至基线, 再给极化液. 在给累加剂量的 CaCl₂ 之前 5 min 给 Mat 0.5 mmol·L⁻¹, 重复测 CaCl₂ 量-效曲线. 结果 Mat 0.5 mmol·L⁻¹ 可以使 CaCl₂ 所致的收缩作用增强, 且 Mat 效应随 Ca²⁺ 浓度递增而加强 (Fig 2A).

Mat 对 KCl 收缩输精管作用的影响 标本在正常 K-H 液中平衡 1 h 后, 累加法给 KCl. 累积浓度依次为 9, 16, 23, 30, 37, 44 mmol·L⁻¹. 用正常 K-H 液冲洗至张力恢复到原有水平. 加入 Mat 1 mmol·L⁻¹, 5 min 后, 重复以上加 KCl 过程. Mat 1 mmol·L⁻¹ 可使 KCl 的去极化作用明显增强 (Fig 2B).

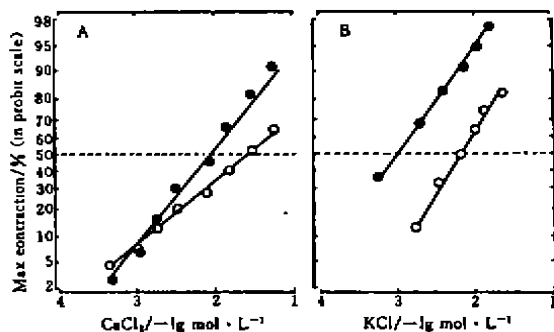


Fig 2. Effects of matrine (Mat) on contraction in isolated *vas deferens* by CaCl₂ (A) and by KCl (B). A: (○) control, (●) Mat 0.5 mmol·L⁻¹ (n=8); B: (○) control, (●) Mat 1 mmol·L⁻¹ (n=7).

DISCUSSION

Mat 加强输精管收缩作用在一定剂量范围内呈线性关系, 剂量过大转为抑制. 从实验可知, Pra 较大剂量 (10 μmol·L⁻¹) 时可抑制 Mat 收缩离体大鼠输精管的作用. 提示 Mat 有较弱的 α₁ 受体兴奋作用. 至于给 Pra 后 Mat 量-效曲线的最大反应压低, 似乎与 Mat

的剂量已达到出现抑制作用有关. Nif 竞争性拮抗 Mat 的作用, 其拮抗指数 pA₂ 为 9.29, 与典型的钙通道激动剂 BAY k 8644 相似^[7]. 唯有最大效应压低项同样可能与 Mat 随剂量增加出现负性肌力作用有关. Ver 与 Mat 呈非竞争性拮抗. 这些均提示 Mat 在一定剂量范围内, 是一钙通道激动剂.

本实验中, Mat (0.5 mmol·L⁻¹) 对高 K⁺ 去极化时的 CaCl₂ 累加量-效反应影响较小 (当 Ca²⁺ 在 2.5 mmol·L⁻¹ 时, 以与 Mat 0.5 mmol·L⁻¹ 对正常 K-H 液即含 Ca²⁺ 量 2.5 mmol·L⁻¹ 时的收缩幅度比较). 可能是因为高 K⁺ 状态下, 钙通道已处于充分开放状态, 所以此时 Mat 的作用无法发挥. 但 Mat 的效应随着浴槽内 Ca²⁺ 浓度递增而加强. 这些都说明 Mat 收缩大鼠输精管的作用是与激活钙通道和细胞外 Ca²⁺ 有联系的. Mat 能增强 KCl 的去极化作用, 原因是 Mat 在 KCl 去极化的基础上进一步开放了钙通道使其去极化作用易化, 还是 Mat 直接抑制钾通道, 协同了 KCl 对细胞膜的去极化作用, 有待研究.

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