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# $\alpha_2$ -肾上腺素受体激活对 3,4-二氨基吡啶诱发去甲肾上腺素释放的调制作用<sup>1</sup>

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**Modulation of 3,4-diaminopyridine-evoked norepinephrine release from rat hippocampal slices by Activation of  $\alpha_2$ -adrenoceptor**

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**ABSTRACT** The effects of the  $\alpha_2$ -adrenoceptor agonist clonidine and antagonist yohimbine were investigated on 3,4-diaminopyridine(DAP)-evoked [<sup>3</sup>H]norepinephrine ([<sup>3</sup>H]NE) release from rat hippocampal slices in the presence and absence of extracellular Ca<sup>2+</sup>. The slices were preincubated with [<sup>3</sup>H]NE and superfused in the presence of desipramine 1  $\mu\text{mol} \cdot \text{L}^{-1}$ . [<sup>3</sup>H]overflow was evoked by addition of DAP 100  $\mu\text{mol} \cdot \text{L}^{-1}$  for 10 min to the superfusion medium.

Clonidine and yohimbine inhibited and enhanced the 3,4-DAP-evoked [<sup>3</sup>H]NE release in a concentration-dependent manner both in the presence and absence of extracellular Ca<sup>2+</sup>. The effect of yohimbine was abolished by clonidine and was additive with the effect of ruthenium red. In the presence of extracellular Ca<sup>2+</sup> the clonidine effect was not altered by addition of  $\omega$ -conotoxin GVIA 0.1  $\mu\text{mol} \cdot \text{L}^{-1}$  or by removal of extracellular Ca<sup>2+</sup>, suggesting that the Ca<sup>2+</sup> entry was not involved in the modulatory mechanisms of DAP-evoked [<sup>3</sup>H]NE release by activation of  $\alpha_2$ -receptor. In the absence of extracellular Ca<sup>2+</sup> the clonidine effect was reduced by the presence of ruthenium red 10  $\mu\text{mol} \cdot \text{L}^{-1}$ , supporting the hypothesis that  $\alpha_2$ -adrenoceptor activation might affect the intracellular mechanism of Ca<sup>2+</sup> homeostasis.

**KEY WORDS** hippocampus; calcium; ruthenium red; norepinephrine; clonidine; yohimbine; aminopyridines

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**提要** 突触前  $\alpha_2$ -肾上腺素能自身受体激动剂 clonidine 和拮抗剂 yohimbine 能抑制和加强 3,4-二氨基吡啶(3,4-DAP)诱发去甲肾上腺素([<sup>3</sup>H]NE)释放, 说明  $\alpha_2$ -自身受体参与 3,4-DAP 诱发 [<sup>3</sup>H]NE 释放的调制. 细胞外无钙时或  $\omega$ -conotoxin 存在时, clonidine 仍能抑制 3,4-DAP 诱发 [<sup>3</sup>H]NE 释放, 说明调制机制与 Ca<sup>2+</sup>通道无关, 可能是通过胞内 Ca<sup>2+</sup>平衡过程而实现的.

**关键词** 海马; 钙; 钨红; 去甲肾上腺素; 可乐定; 育亨宾; 氨基吡啶类

突触前  $\alpha_2$ -肾上腺素能自身受体激活后能抑制 NE 的释放, 但对其作用机制尚得不到一致的结论. 主要的几种假设认为:  $\alpha_2$ -自身受体激活后, 影响 Ca<sup>2+</sup>通道, 减少细胞外 Ca<sup>2+</sup>内流;  $\alpha_2$ -自身受体激活后, 与受体相偶联的 K<sup>+</sup>通道开放而使 K<sup>+</sup>外流, 因而神经末梢可兴奋膜处于超极化状态; 或  $\alpha_2$ -受体激活后影响细胞内钙的平衡机制<sup>[1]</sup>. 由于我们所采用的 DAP 诱发 NE 释放在细胞外无钙条件下亦能进行<sup>[2]</sup>, 因此, 我们可以研究:  $\alpha_2$ -肾上腺素能自身受体的作用机制是否通过影响 Ca<sup>2+</sup>通道, 抑制细胞外 Ca<sup>2+</sup>内流入轴突末梢内所致.

## MATERIALS AND METHODS

**药品和试剂** [<sup>3</sup>H]去甲肾上腺素(1-(7,8-[<sup>3</sup>H]norepinephrine, [<sup>3</sup>H]NE, Amersham); 3,4-二氨基吡啶(3,4-diaminopyridine, 3,4-DAP), desipramine HCl, 育亨宾(yohimbine HCl), 可乐定(clonidine HCl) (Sigma),  $\omega$ -conotoxin GVIA (Peninsula Laboratories); 钨红(ruthenium red, Merck); Soluene 350, Ultima-gold, Hionic-fluor (Packard)

Sprague-Dawley 大鼠,  $\uparrow$ , 体重 250  $\pm$  s 25 g,

断头,取出全脑,投入4℃生理溶液,在6-8℃下分离海马,用McIlwain组织切片机,沿板状器方向制备厚0.4 mm的脑片,用生理溶液淋洗后,加入生理溶液2 ml,含 $[^3\text{H}]\text{NE}$   $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ 、 $1.6 \text{TBq} \cdot \text{mmol}^{-1}$ 、37℃保温30 min,再用生理溶液淋洗3次,将脑片随机转入容量1 ml的灌流小室,每室1片,以 $0.7 \text{ ml} \cdot \text{min}^{-1}$ 流速进行表面灌流,生理溶液成分( $\text{mmol} \cdot \text{L}^{-1}$ ): NaCl 118, KCl 4.8,  $\text{CaCl}_2$  1.3,  $\text{MgSO}_4$  1.2,  $\text{NaHCO}_3$  25,  $\text{KH}_2\text{PO}_4$  1.2, 葡萄糖 11, 抗坏血酸 0.57, EDTA二钠盐 0.03, 用95%  $\text{O}_2$  + 5%  $\text{CO}_2$  饱和,加NaOH调pH至7.4,灌流液中另加desipramine  $1 \mu\text{mol} \cdot \text{L}^{-1}$ ,灌流45 min后,将流出液直接收集入闪烁杯内,每5 min 1份,加闪烁液Ultima-gold 4 ml,用液体闪烁计数仪测 $[^3\text{H}]$ 含量,脑片用Soluvue 350 0.25 ml溶解后,加入Hionic fluor 5 ml,然后测定 $[^3\text{H}]$ 含量。

在灌流开始后60 min引入DAP  $100 \mu\text{mol} \cdot \text{L}^{-1}$ 持续10 min以诱发 $[^3\text{H}]\text{NE}$ 释放(色谱分析证明90%以上是 $[^3\text{H}]\text{NE}$ ),通常在刺激之前15 min加入待测药物,以测试其对诱发释放的影响,每次同时进行对照实验。

被测药物的作用以计算刺激所诱发释放的 $[^3\text{H}]$ 包括刺激开始后10份样品中 $[^3\text{H}]$ 含量减去基础释放量)占脑片 $[^3\text{H}]$ 总含量的百分率来评价,所有结果均以 $\bar{x} \pm s$ 表示,用t检验测定组间差别的显著性<sup>[3,4]</sup>。

## RESULTS

**1 育亨宾和可乐定对DAP诱发 $[^3\text{H}]\text{NE}$ 释放的影响** 在细胞外有钙或无钙条件下, $\alpha_2$ -自身受体拮抗剂育亨宾均能显著地加强DAP诱发释放NE,从 $0.01-10 \mu\text{mol} \cdot \text{L}^{-1}$ 呈浓度依赖关系,在细胞外有钙条件下育亨宾 $10 \mu\text{mol} \cdot \text{L}^{-1}$ 加强DAP诱发 $[^3\text{H}]\text{NE}$ 释放为对照组的1.85倍,而在无钙条件下,同一浓度的育亨宾加强 $[^3\text{H}]\text{NE}$ 释放为对照组的1.97倍,同样, $\alpha_2$ -自身受体的激动剂可乐定无论在细胞外有钙或无钙条件下均能抑制3,4-DAP诱发释放 $[^3\text{H}]\text{NE}$ ,从 $0.001-10 \mu\text{mol} \cdot \text{L}^{-1}$

呈浓度依赖关系,在细胞外有钙条件下,可乐定 $10 \mu\text{mol} \cdot \text{L}^{-1}$ 抑制 $[^3\text{H}]\text{NE}$ 释放为对照组的44%,而在无钙条件下,同一浓度的可乐定抑制 $[^3\text{H}]\text{NE}$ 释放为对照组的19% (Tab 1)。

**Tab 1. Effects of yohimbine and clonidine on 3,4-diaminopyridine-evoked  $[^3\text{H}]\text{NE}$  release.** Rat hippocampal slices were preincubated with  $[^3\text{H}]\text{NE}$  and superfused with medium containing desipramine  $1 \mu\text{mol} \cdot \text{L}^{-1}$  and  $\text{Ca}^{2+}$  0 or  $1.3 \text{ mmol} \cdot \text{L}^{-1}$ . Slices were stimulated for 10 min by adding 3,4-DAP  $100 \mu\text{mol} \cdot \text{L}^{-1}$  after 60 min of superfusion. Yohimbine or clonidine was added 15 min before stimulation.  $\bar{x} \pm s$ ,  $n = 4-10$  slices from 3 rats.

	Evoked overflow of $[^3\text{H}]$ (% of tissue- $[^3\text{H}]$ ) + $\text{Ca}^{2+}$	- $\text{Ca}^{2+}$
Clonidine / $\mu\text{mol} \cdot \text{L}^{-1}$		
0	$7.95 \pm 0.20$	$4.50 \pm 0.31$
0.001		$4.15 \pm 0.21$
0.01	$7.49 \pm 0.40$	$1.88 \pm 0.21$
0.1	$4.08 \pm 0.28$	$1.22 \pm 0.12$
1	$3.26 \pm 0.19$	$0.75 \pm 0.09$
10	$3.30 \pm 0.24$	$0.69 \pm 0.09$
Yohimbine / $\mu\text{mol} \cdot \text{L}^{-1}$		
0	$7.95 \pm 0.20$	$4.21 \pm 0.15$
0.01	$12 \pm 1.10$	$5.00 \pm 0.30$
0.1	$19.00 \pm 1.00$	$7.12 \pm 0.62$
1	$22.21 \pm 1.90$	$12.21 \pm 0.81$
10	$25.34 \pm 2.16$	$13.62 \pm 1.05$

**2 当可乐定或钉红存在时,育亨宾对DAP诱发 $[^3\text{H}]\text{NE}$ 释放的影响** 当可乐定 $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ 存在时,育亨宾 $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ 对DAP诱发释放 $[^3\text{H}]\text{NE}$ 的易化作用被抵消,钉红 $10 \mu\text{mol} \cdot \text{L}^{-1}$ 能加强DAP诱发 $[^3\text{H}]\text{NE}$ 释放,当钉红 $10 \mu\text{mol} \cdot \text{L}^{-1}$ 存在时,育亨宾 $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ 对DAP诱发 $[^3\text{H}]\text{NE}$ 释放的加强作用不发生显著性变化,它和钉红的作用呈相加现象(Fig 1)。

**3  $\omega$ -Conotoxin GVIA存在时,可乐定对DAP诱发 $[^3\text{H}]\text{NE}$ 释放的抑制作用** 从Tab 2可见在细胞外液含 $\text{Ca}^{2+}$   $1.3 \text{ mmol} \cdot \text{L}^{-1}$ 时, $\omega$ -conotoxin  $0.1 \mu\text{mol} \cdot \text{L}^{-1}$ 明显抑制DAP诱

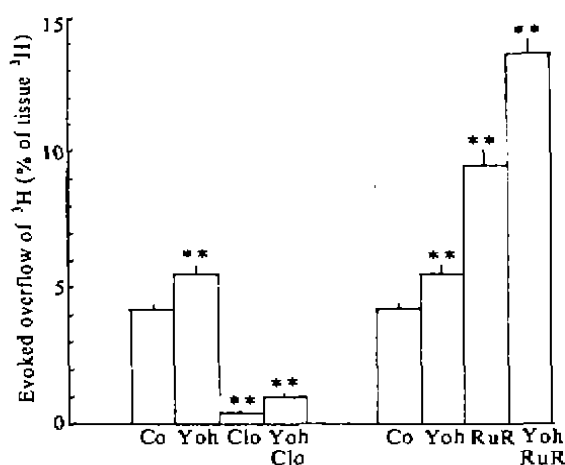


Fig 1. Effects of yohimbine with or without clonidine or ruthenium red on <sup>3</sup>H]NE release evoked by 3,4-DAP in the absence of extracellular Ca<sup>2+</sup>. Slices were preincubated with <sup>3</sup>H]NE and superfused with Ca<sup>2+</sup>-free medium containing EGTA 1 mmol · L<sup>-1</sup> and desipramine 1 μmol · L<sup>-1</sup>. Slices were stimulated for 10 min by addition of 3,4-DAP 100 μmol · L<sup>-1</sup> after 60 min of superfusion. yohimbine (Yoh) 0.1 μmol · L<sup>-1</sup>, clonidine (Clo) 0.1 μmol · L<sup>-1</sup> or ruthenium red (RuR) 10 μmol · L<sup>-1</sup> was added to the medium 15 min before stimulation,  $\bar{x} \pm s$ , n=4-6 slices from 2 rats, \*\*P<0.05, \*\*\*P<0.01 vs controls (Co).

发<sup>3</sup>H]NE 释放(P<0.05)。当 ω-conotoxin 0.1 μmol · L<sup>-1</sup> 存在时, α<sub>2</sub>-自身受体激动剂可乐定仍能显著地抑制 DAP 诱发<sup>3</sup>H]NE 释放, 该抑制作用仍呈浓度依赖关系, 可乐定 10 μmol · L<sup>-1</sup> 对<sup>3</sup>H]NE 释放的抑制仍可达 43%, 与在有钙条件下, ω-conotoxin 不存在时(Fig 1)可乐定 10 μmol · L<sup>-1</sup> 抑制<sup>3</sup>H]NE 释放 44%相比, 无显著差别。

4 钉红存在时, 可乐定对 DAP 诱发<sup>3</sup>H]NE 释放的抑制作用 在无钙灌流液中加入 EGTA 1 mmol · L<sup>-1</sup>, 使细胞外液中的游离钙浓度下降到 10<sup>-9</sup> mol · L<sup>-1</sup>, 这时可乐定对 3,4-DAP 诱发<sup>3</sup>H]NE 释放的抑制作用随可乐定浓度的增加而增强。当钉红 10 μmol · L<sup>-1</sup> 存在时, 可乐定对 DAP 诱发<sup>3</sup>H]NE 释放的抑制作用虽仍呈浓度依赖关系, 但却大大减弱了。

在对照组, 可乐定 10 μmol · L<sup>-1</sup> 抑制<sup>3</sup>H]NE 释放到 13%, 而在钉红组, 同样浓度的可乐定只抑制<sup>3</sup>H]NE 释放到对照组的 43% (Tab 3)。

Tab 2. Effect of clonidine on DAP-evoked <sup>3</sup>H]NE release in the presence of ω-conotoxin GVIA. Slices were preincubated with <sup>3</sup>H]NE and superfused with a medium containing Ca<sup>2+</sup> 1.3 mmol · L<sup>-1</sup> and desipramine 1 μmol · L<sup>-1</sup>. During superfusion the slices were stimulated for 10 min by addition of 3,4-DAP 100 μmol · L<sup>-1</sup> to the medium. Clonidine and ω-conotoxin GVIA 0.1 μmol · L<sup>-1</sup> were added 15 min before stimulation,  $\bar{x} \pm s$ , n=4-6 slices from 2 rats. \*\*P<0.05, \*\*\*P<0.01 vs control.

ω-conotoxin/ μmol · L <sup>-1</sup>	Clonidine/ μmol · L <sup>-1</sup>	Evoked overflow of <sup>3</sup> H (% of tissue <sup>3</sup> H)
0	0	6.1 ± 0.2
0.1	0	3.8 ± 0.3**
0.1	0.01	2.5 ± 0.1**
0.1	0.1	2.2 ± 0.1***
0.1	1	2.0 ± 0.2***
0.1	10	1.8 ± 0.1***

Tab 3. Effect of clonidine with or without ruthenium red on DAP-evoked <sup>3</sup>H]NE release in the absence of extracellular Ca<sup>2+</sup>. Hippocampal slices were preincubated with <sup>3</sup>H]NE and superfused with Ca<sup>2+</sup>-free medium containing EGTA 1 mmol · L<sup>-1</sup> and desipramine 1 μmol · L<sup>-1</sup>. During superfusion the slices were stimulated for 10 min by addition of DAP 100 μmol · L<sup>-1</sup>. Clonidine and ruthenium red 10 μmol · L<sup>-1</sup> were added 15 min before stimulation.  $\bar{x} \pm s$ .

Clonidine/ μmol · L <sup>-1</sup>	Evoked overflow of <sup>3</sup> H (% of tissue- <sup>3</sup> H)			
	Control	n	Ruthenium red/ 10 μmol · L <sup>-1</sup>	n
0	5.00 ± 0.32	6	11.7 ± 0.4	6
0.001	4.15 ± 0.21	4	11.7 ± 0.5	4
0.01	1.9 ± 0.3	4	8.11 ± 0.20	4
0.1	0.75 ± 0.09	4	5.4 ± 0.4	4
1	0.69 ± 0.09	4	5.0 ± 0.3	4

DISCUSSION

突触前 α<sub>2</sub>-肾上腺素能自身受体对去甲肾

上腺素释放的负反馈调制机制现有 3 种假设: (1) 突触前  $\alpha_2$ -自身受体激活直接影响钙离子通道<sup>1)</sup>, 减少细胞外钙离子进入膜内从而减少去甲肾上腺素释放. (2) 突触前  $\alpha_2$ -自身受体激活从而激活  $K^+$ 通道,  $K^+$ 外流, 使神经末梢膜处于超极化状态, 继发性地减少  $Ca^{2+}$ 进入细胞膜并抑制该神经递质的释放<sup>6)</sup>. (3) 突触前  $\alpha_2$ -自身受体激活影响细胞内钙离子的平衡过程, 如游离钙的释放或隐退到细胞浆内的钙池( $Ca^{2+}$  pools)<sup>7)</sup>.

本实验结果显示, 突触前  $\alpha_2$ -自身受体的拮抗剂育亨宾和激动剂可乐定能显著地加强和抑制 DAP 诱发释放 [ $^3H$ ]NE, 说明  $\alpha_2$ -自身受体参与 DAP 诱发 [ $^3H$ ]NE 释放模型的调制. 我们的结果不支持第 1 种假设, 因为在细胞外无钙时, 或在电压依赖性  $N$ -型钙离子通道阻断剂  $\omega$ -conotoxin<sup>8)</sup>存在时, 没有  $Ca^{2+}$ 进入膜内,  $\alpha_2$ -自身受体的激动剂可乐定仍能显著地抑制 DAP 诱发 [ $^3H$ ]NE 释放. 对于第 2 种假设我们不能完全排除, 尽管 DAP 是  $K^+$ 通道的阻断剂, 但它仅仅作用在  $I_A$ <sup>12,9)</sup>或  $I_D$ <sup>11)</sup>电流, 对其它种类的钾电流 DAP 并没有作用, 因此  $\alpha_2$ -自身受体激动后, 仍可能激活其它种类的  $K^+$ 电流, 使膜处于超极化状态, 这样 DAP 引起膜的去极化只在发生动作电位的阈值以下, 因此 DAP 诱发的 [ $^3H$ ]NE 释放仍可能被抑制. 本实验结果对第 3 种假设给予进一步的支持, 我们以前的工作已证明, 在 3,4-DAP 诱发 [ $^3H$ ]NE 释放机制中, 细胞内  $Ca^{2+}$ 释放比细胞外  $Ca^{2+}$ 经电压依赖性  $Ca^{2+}$ 通道进入细胞膜内更为重要<sup>12)</sup>. 从 Tab 3 可以看到, 在细胞外无钙条件下, 使用  $Ca^{2+}$ 重摄取阻断剂钉红<sup>11)</sup>抑制细胞内游离钙重新被摄入线粒体而使胞浆游离  $Ca^{2+}$ 浓度升高时,  $\alpha_2$ -自身受体激动剂可乐定对 DAP 诱发 [ $^3H$ ]NE 释

放作用明显减弱, 说明可乐定对细胞内游离钙平衡过程的影响部分地被钉红抵消了.

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