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吡那地尔对 5-HT₃ 受体介导的 离体豚鼠回肠收缩反应的抑制

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关键词 血清素受体; 吡那地尔; 回肠; 肠肌丛;
卡巴胆碱; GR65630

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收缩反应

目的: 研究钾通道开放剂吡那地尔对 5-HT₃ 受体介导的离体豚鼠回肠(GPI)收缩反应的影响。方法: 以等长换能器记录 GPI 收缩反应, 以 [³H]GR65630 结合试验检测大鼠内嗅皮层 5-HT₃ 受体的结合特性。结果: (1) 2-甲基-5-HT 及 5-HT 以剂量依赖方式引起 GPI 的收缩; 托品色创竞争性抑制此反应。(2) 吡那地尔以剂量依赖方式抑制 2-甲基-5-HT 和 5-HT 引起的 GPI 收缩, 并增强托品色创或 Benesetron 对 5-HT 诱发 GPI 收缩的抑制作用, 但不影响卡巴胆碱引起的 GPI 收缩; 吡那地尔对大鼠内嗅皮层 5-HT₃ 受体与 [³H]GR65630 的结合无影响。结论: 吡那地尔可能通过激活突触前神经元 ATP 敏感钾通道抑制由 5-HT₃ 受体介导的 GPI 收缩反应。

Effects of long-term application of dopamine HCl on dopamine agonist-induced cAMP production in rat renal cortex¹

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KEY WORD dopamine receptors; kidney cortex; dopamine; cyclic AMP; dopamine agents; adenylyl cyclase; Sch-23390; domperidone

AIM: To study the effects of long-term application of dopamine HCl (DA) on the functional changes of dopamine receptor subtypes coupled to adenylyl cyclase in rat renal cortex. **METHODS:** cAMP levels were measured by radioimmunoassay as an index of dopamine receptor function. **RESULTS:** Injection of DA (30 mg · kg⁻¹ · d⁻¹, ip, 30 d) reduced the fenoldopam (Fen) (100 μmol · L⁻¹)-induced increments of cAMP production from the control group of +1.26 ± 0.04 to the DA-treated group of +0.63 ± 0.22 nmol · min⁻¹/g tissue and the propyl-butyl-

dopamine (PBDA) (100 μmol · L⁻¹)-induced decrements of cAMP production in the presence of Sch-23390 (Sch) from the control group of -0.38 ± 0.18 to the DA-treated group of -0.11 ± 0.08 nmol · min⁻¹/g tissue with, however, comparable percentile changes for the 2 groups. Sch blocked both Fen- and PBDA-induced increase in cAMP production, while domperidone (Dom) blocked the decreasing effects of PBDA on cAMP accumulation in the presence of Sch. **CONCLUSION:** Long-term application of DA produced a marked "down regulation" of both DA₁ and DA₂ receptors in rat renal cortex with, however, the responsiveness of the remaining receptors unchanged.

Dopamine HCl (DA) agonists have been extensively used in the treatment of cardiovascular and kidney disease^[1,2]. But, long-term application of the drugs may result in the decrease of drug effects in patients and animals, indicating the occurrence of some

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changes in receptor response^{3,4}). Although the short-term exposure of tissue cells to DA agonists induced desensitization of dopamine-1 receptors^{5,6}, no evidence is available regarding the dopamine-1 receptor desensitization induced by long-term treatment with DA agonists in intact bodies which is more close to the clinical situation. So, in this study we examined the effects of long-term application of DA on the function of kidney DA₁ and DA₂ receptor subtypes by measuring cAMP production as an index of DA receptor function.

MATERIALS AND METHODS

Tissue preparation Wistar rats (27 \hat{c} , weighing 150 \pm 5 g) were randomly divided into 2 groups: DA group ($n = 15$) was medicated with DA HCl (30 mg \cdot kg⁻¹ \cdot d⁻¹ \times 30 d ip) and control group ($n = 12$) was injected with saline. Rats were decapitated and the whole kidney was transferred to ice-cold Krebs-bicarbonate solution gassed with 95 % O₂ + 5 % CO₂. A strip of renal cortex (0.1 g) was suspended in organ bath chambers containing modified Krebs-bicarbonate solution: NaCl 118, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25, and glucose 11 (mmol \cdot L⁻¹). Propranolol (10 μ mol \cdot L⁻¹) was added to block β -adrenoceptor effects. Bath solution was maintained at 37 $^{\circ}$ C, pH 7.4. Tissues were allowed to equilibrate for 1 h and the solution was changed every 20 min. DA agonists (0.1 – 100 μ mol \cdot L⁻¹) were added and incubated for 20 min. The selective DA subtype antagonist (Sch-23390 or domperidone, 10 μ mol \cdot L⁻¹) were added at 20 min before DA agonists. The reaction was terminated by trichloroacetic acid (1 mol \cdot L⁻¹ 500 μ L). The tissue homogenates were spun at 15 \times g, 4 $^{\circ}$ C for 15 min. The supernatants were neutralized by KOH (2 mol \cdot L⁻¹) and spun at 7 \times g for 10 min. The supernatant 50 μ L was used for cAMP radioimmunoassay.

cAMP assay To increase the sensitivity of cAMP radioimmunoassay, samples were acetylated before assay⁷. cAMP was determined by a radioimmunoassay kit made by Shanghai Second Medical University. Acetic buffer 100 μ L,

acetylated reagents 10 μ L, [³H] cAMP 9 GBq \cdot L⁻¹, and anti-cAMP serum (1:20000) 100 μ L were added sequentially. After incubation at 4 $^{\circ}$ C over night, the reaction was terminated by 1 mL ice-cold phosphate buffer 20 mmol \cdot L⁻¹, pH 6.0 followed by vacuum filtration through Millipore membrane, which was then stoven at 80 $^{\circ}$ C for 30 min and assayed for cAMP using liquid scintillation counter (LS-3301, Beckman). Measured cAMP was expressed as nmol of cAMP accumulation per g per min.

Chemicals Fenoldopam (Fen, SK&F 82526, SmithKline Beecham, USA), Propyl-butyl-dopamine (PBDA, Sloan-Kettering Cancer Center, USA), Sch-23390 (Sch, Schering-Plough, USA), domperidone (Janssen Pharmaceutica Inc, USA), dopamine HCl and propranolol HCl (Beijing Pharmaceutical Factory). All other chemicals were AR.

Statistical analysis The values are presented as $\bar{x} \pm s$. Student's paired or non-paired t tests were used as appropriate.

RESULTS

Basal cAMP accumulation Under the same experimental condition, in DA-treated group the basal cAMP accumulation was 1.44 ± 0.22 nmol \cdot min⁻¹/g tissue, which was much lower than that of 2.31 ± 0.12 nmol \cdot min⁻¹/g tissue in control group with $P < 0.01$ (Tab 1).

Effects of Fen Various concentrations of Fen (0.1 – 100 μ mol \cdot L⁻¹), a selective DA₁ subtype agonist, stimulated the cAMP production in a dose-dependent manner in both DA-treated and control groups (Fig 1). At a dose of 10 μ mol \cdot L⁻¹, Fen increased the cAMP level from the basal value of 1.44 ± 0.22 to 2.07 ± 0.14 nmol \cdot min⁻¹/g tissue in DA-treated group, while in control group it increased the cAMP level from the basal value of 2.31 ± 0.12 to 3.58 ± 0.07 nmol \cdot min⁻¹/g tissue (Tab 1). Although the increase extent in terms of absolute value

Tab 1. Effects of long-term application of DA on dopamine agonist-induced cAMP accumulation in rat renal cortex (μ mol \cdot min⁻¹/g tissue). $n = 3$ experiments in duplicate. $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$, ^c $P < 0.01$ vs basal.

Stimulators/ 10 μ mol \cdot L ⁻¹	Values	Control	DA-treated for 30 d	
		Changes	Values	Changes
Basal	2.31 ± 0.12		1.44 ± 0.22	
Fen	3.58 ± 0.07^c	$+1.26 \pm 0.07$	2.07 ± 0.14^c	$+0.63 \pm 0.47$
Fen + Sch	2.34 ± 0.05^d	$+0.05 \pm 0.05^c$	1.48 ± 0.20^a	$+0.09 \pm 0.04$
PBDA	2.89 ± 0.07^b	$+0.63 \pm 0.03$	1.88 ± 0.08^c	$+0.45 \pm 0.30$
Dom + PBDA	3.12 ± 0.03^c	$+0.81 \pm 0.14$	1.99 ± 0.02^b	$+0.55 \pm 0.21$
PBDA + Sch	1.97 ± 0.31^b	-0.38 ± 0.31	1.27 ± 0.20^b	-0.11 ± 0.13

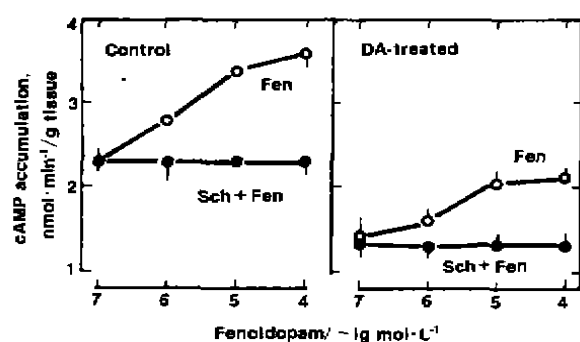


Fig 1. Effects of Fen and Fen + Sch-23390 on cAMP accumulation in rat renal cortex. Rats were administered with (DA-treated) or without (control) DA for 30 d. Fen: fenoldopam, Sch: Sch-23390. $n = 3$ experiments in duplicate. $\bar{x} \pm s$.

was much smaller in DA-treated group than in control group, the percent increases were comparable for the two groups (Tab 2).

Tab 2. Effects of long-term application of DA on dopamine agonist-induced % changes in cAMP accumulation in rat renal cortex. Stimulators = $0.1 \mu\text{mol} \cdot \text{L}^{-1}$. $n = 3$ experiments in duplicate. $\bar{x} \pm s$. $^{\text{a}}P > 0.05$ vs control for both Fenoldopam and PBDA + Sch.

Fen		PBDA + Sch	
Control/%	DA-treated/%	Control/%	DA-treated/%
3.0 ± 0.6	$1.9 \pm 2.0^{\text{a}}$	-1.6 ± 0.5	$-1.8 \pm 2.4^{\text{a}}$
7.9 ± 3.1	$10.0 \pm 1.8^{\text{a}}$	-8.1 ± 1.3	$-6.0 \pm 2.1^{\text{a}}$
54.0 ± 1.3	$48.1 \pm 6.7^{\text{a}}$	-17.9 ± 2.3	$-19.0 \pm 0.5^{\text{a}}$
57.0 ± 0.7	$53.7 \pm 2.1^{\text{a}}$	-14.9 ± 1.6	$-14.0 \pm 2.4^{\text{a}}$

Effects of PBDA At the same dose range of $0.1 - 100 \mu\text{mol} \cdot \text{L}^{-1}$, PBDA, a selective DA_2 subtype agonist, also induced a dose-related increase in cAMP production in both DA-treated and control group albeit to a lesser degree (Fig 2). At the dose of $10 \mu\text{mol} \cdot \text{L}^{-1}$, PBDA induced the cAMP accumulation from the basal value of 1.44 ± 0.22 to $1.88 \pm 0.08 \text{ nmol} \cdot \text{min}^{-1}/\text{g}$ tissue in DA-treated group and from the basal 2.31 ± 0.12 to $2.89 \pm 0.07 \text{ nmol} \cdot \text{min}^{-1}/\text{g}$ tissue in control group (Tab 1). Again the increasing extent in terms of absolute value was smaller in DA-treated group than that in control group with, however, the value of percent increases being in proximity to each other for the two groups.

Effects of antagonists The effects of the

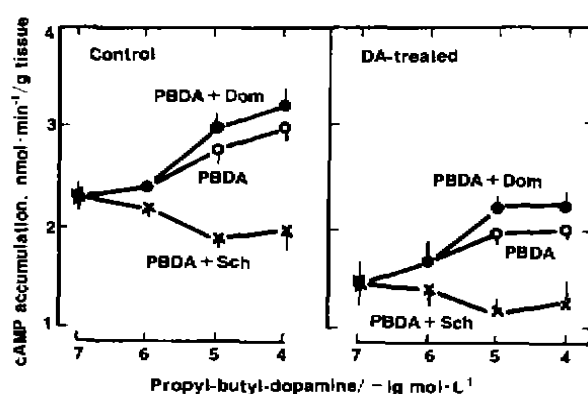


Fig 2. Effects of PBDA, PBDA + Sch and PBDA + Dom on cAMP accumulation in rat renal cortex. PBDA: propyl-butyl-dopamine, Dom: domperidone, Sch: Sch-23390.

selective DA_1 -receptor antagonist, Sch, and the selective DA_2 -receptor antagonist, domperidone, on fenoldopam- and PBDA-induced cAMP accumulation were summarized in Tab 1. It is evident that Sch blocked both fenoldopam- and PBDA-induced cAMP increase in both control and DA-treated groups, while domperidone slightly increased the PBDA-induced cAMP production. It is noteworthy that in the presence of Sch, PBDA induced a dose-related decrease, but not increase, in cAMP accumulation in both groups (Tab 1, 2).

DISCUSSION

Our results showed that after long-term application of DA (a combined DA_1 and DA_2 receptor agonist), the extent of changes in cAMP accumulation in rat renal cortex including the increasing extent induced by Fen and the decreasing extent induced by PBDA in the presence of Sch were markedly attenuated suggesting that the DA receptors (both DA_1 and DA_2 subtypes) are readily desensitized in that tissue. These results were supported by the observations of Bates *et al*⁽⁸⁾ and Kinoshita *et al*⁽⁶⁾ showing that the DA_1 -receptor binding sites were reduced by pretreatment with dopamine or dopamine- β -hydroxylase inhibitor.

DA_1 receptors are linked to the stimulation of adenylyl cyclase (AC) activity, while DA_2 receptors are associated with inhibition of cAMP generating system⁽⁹⁾. In this study, fenoldopam, a selective DA_1 receptor agonist, increased the cAMP accumulation in both groups. PBDA is nominally considered as a

selective DA₂ agonist, however, it also has some stimulatory action on DA₁ receptors^[10]. Therefore, PBDA, when used alone, also increased the cAMP production slightly and its inhibitory effects on cAMP production via DA₂ receptors could only be revealed in the presence of selective DA₁ receptor antagonist, Sch. The stimulatory effects of both fenoldopam and PBDA on cAMP production were completely blocked by Sch, while the inhibitory effects of PBDA on cAMP production were abolished by the selective DA₂ receptor antagonist, domperidone. Thus, our results demonstrated the existence of both DA₁ and DA₂ receptors in the rat renal cortex.

In this study, the extent of increase in cAMP production induced by Fen and decrease in cAMP accumulation induced by PBDA in the presence of Sch were considerably attenuated in terms of absolute value in the DA-treated group after long-term application of DA as compared with the control group. However, the extent of percent changes were basically identical for the two groups. This suggested that the number of receptor sites was down-regulated by prolonged exposure to receptor agonists with, however, the remaining receptors kept functionally unchanged. This is in agreement with the findings of Balmforth *et al*^[11] who have done similar experiments on human astrocytoma cells.

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长期给予大鼠盐酸多巴胺对多巴胺受体激动剂诱导的肾皮质 cAMP 蓄积量的影响

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关键词 多巴胺受体; 肾皮质; 多巴胺; 环腺苷一磷酸; 多巴胺药; 腺苷酸环化酶; Sch-23390; 多潘立酮 cAMP

目的: 观察长期给予盐酸多巴胺对大鼠肾皮质多巴胺受体亚型所介导的腺苷酸环化酶活性的影响。方法: 用放免分析法测定 cAMP 含量, 作为反映多巴胺受体功能的指标。结果: 长期给予盐酸多巴胺可显著减少肾皮质由非诺多泮引起的 cAMP 增加的量和在 Sch-23390 存在下由 PBDA 引起的 cAMP 降低的量, 但其变量百分比则与对照组无显著差异。Sch-23390 可阻断由非诺多泮和 PBDA 引起的 cAMP 的增加, 而多潘立酮可阻断在 Sch-23390 存在下由 PBDA 引起的 cAMP 的降低。结论: 长期应用多巴胺可使大鼠肾皮质的 DA₁ 和 DA₂ 受体均发生明显的“下调”, 但余留受体的反应性不变。

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