

Protection of dopaminergic antagonists against anoxia-induced inhibition of Ca^{2+} -calmodulin dependent protein kinase II activity in rat brain¹

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KEY WORDS dopamine; Ca^{2+} -calmodulin dependent protein kinase; anoxia; Sch-23390; domperidone; hippocampus; corpus striatum; adenosine triphosphate

ABSTRACT

AIM: To study the effect of dopamine receptor antagonists on anoxia-induced inhibition of Ca^{2+} -calmodulin dependent protein kinase II (CCDPK II) activity in rat hippocampus and striatum.

METHODS: Using the rat hippocampal and striatal slices under 95 % N_2 + 5 % CO_2 , the activity of CCDPK II was examined by ³²P-incorporation.

RESULTS: Under anoxia for 30 min, the CCDPK II activity decreased to 29.2 % and 27.0 % of the control in rat hippocampal and striatal slices, respectively. Preincubation with Sch-23390 (a specific D_1 -like dopamine receptor antagonist), or domperidone (a specific D_2 -like dopamine receptor antagonist), resulted in a concentration-dependent attenuation of the anoxia-induced inhibition of CCDPK II activity which was preserved up to about 60 %. **CONCLUSION:** Dopamine receptor stimulation is involved in anoxia-induced inhibition of CCDPK II activity in rat hippocampus and striatum.

INTRODUCTION

The most vulnerable brain regions to ischemic damage are hippocampus and striatum. Using *in vivo*

microdialysis, many experiments have demonstrated that there is a massive release of excitatory amino acids (EAA), including glutamate and aspartate, and dopamine in the hippocampus and striatum during cerebral ischemia. It is now generally accepted that EAA is an essential factor in mediating ischemic damage through an excitotoxic mechanism. Dopaminergic deafferentation by injection of 6-hydroxydopamine into the substantia nigra (SN) or medial forebrain bundle (MFB) could attenuate the severity of ischemic and excitotoxic cerebral damage in the hippocampus and striatum^[1-3], which suggested that the excessive release of dopamine mediated a neurotoxic effect during cerebral ischemia.

Dopamine receptors modulate intracellular calcium levels^[4]. Calcium has a pivotal effect on ischemic damage. Elevation of intracellular calcium concentration could activate the calcium-dependent enzyme, such as Ca^{2+} -calmodulin dependent protein kinase II (CCDPK II), etc, and result in neuronal damage.

The multifunctional CCDPK II, enriched in neural tissues, is a sensitive enzyme to ischemia. Previously, we reported that transient ischemia could result in the time-dependent significant inhibition of CCDPK II activity, and the inhibition of the enzyme activity contributed to neuronal damage^[5]. In this paper, the effects of dopamine receptor antagonists on the anoxia-induced inhibition of CCDPK II activity were investigated to evaluate the relationship between dopamine and CCDPK II activity during cerebral ischemia and the mechanism of dopaminergic neurotoxicity.

MATERIALS AND METHODS

Materials R(+)-Sch-23390 hydrochloride and

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domperidone were purchased from Research Biochemicals International Co (RBI), USA. Sch-23390 was dissolved in a small amount of distilled water and domperidone was dissolved in 1 % lactate, and diluted with glucose-free Krebs-Ringer solution immediately before use. [γ - 32 P]ATP was purchased from Yuhui Biological and Medical Engineering Co, Beijing; ATP from Sigma; phenylmethane-sulfonylfluorid (PMSF) and mercapto ethenol (β -ME) from E Merck. Other reagents were of AR.

Hippocampal and striatal slices preparation

Male Sprague-Dawley rats (Grade II, Certificate No D02-49-2, purchased from Sippr-BK Experimental Animal Ltd Co, Shanghai), weighing 160 - 200 g, were decapitated and the brains were placed in ice-cold Krebs-Ringer solution; NaCl 122, KCl 3.1, KH_2PO_4 0.4, MgSO_4 1.2, CaCl_2 1.3, NaHCO_3 25, glucose 10 $\text{mmol} \cdot \text{L}^{-1}$; pH 7.4, equilibrated with 95 % O_2 + 5 % CO_2 . Parasagittal hippocampal slices (350 μm) and striatal slices (300 μm) were prepared with a McIlwain tissue chopper and preincubated in normal Krebs-Ringer solution at 36 $^\circ\text{C}$ for 90 min, continuously bubbled with 95 % O_2 + 5 % CO_2 .

Anoxia and drug application Following preincubation, slices were transferred to glucose-free Krebs-Ringer solution for 30 min, continuously bubbled with 95 % N_2 + 5 % CO_2 . DA receptor antagonists were added 15 min before anoxia. After experiments, the slices were quickly removed and frozen in liquid nitrogen until use.

Assay for CCDPK II activity The slices were homogenized in a glass homogenizer on ice with ice-cold homogenization buffer; Tris-HCl 20, edetic acid 2, NaF 2, PMSF 0.5, β -ME 10 $\text{mmol} \cdot \text{L}^{-1}$; pH 7.5. The homogenate was spun at 10 000 $\times g$ at 4 $^\circ\text{C}$ for 5 min, and the supernatant was assayed for Ca^{2+} -calmodulin dependent activity of CCDPK II by the method of ^{32}P -incorporation^[5]. The phosphorylation reaction was initiated by the addition of [γ - ^{32}P]ATP and incubated at 37 $^\circ\text{C}$ for 5 min. Twenty microliter reaction mixture was spotted on 2 cm \times 2 cm filter paper squares. The paper was immediately immersed in ice-cold H_3PO_4 75 $\text{mmol} \cdot \text{L}^{-1}$ to stop the reaction. The paper was then washed three times (10 min each) in ice-cold H_3PO_4 75 $\text{mmol} \cdot \text{L}^{-1}$, rinsed with EtOH (100 %), dried with a hair dryer, and immersed in scintillant. The radioactivity was measured by a liquid

scintillation spectrometer (LS 6500, Beckman). Protein concentration of the supernatant was determined with bovine serum albumin (BSA) as standard^[6]. The CCDPK II activity was expressed in the amount of ^{32}P -incorporation ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$).

Statistical analysis Values were expressed as $\bar{x} \pm s$. The statistical analysis of the data was performed by ANOVA followed by the Duncan's new multiple range method or Newman-Keuls test.

RESULTS

Anoxia for 30 min resulted in significant inhibition of CCDPK II activity in rat hippocampal and striatal slices, and the enzyme activity fell to 29.2 % and 27.0 % of the control, respectively.

Preincubation with Sch-23390, a specific D_1 -like dopamine receptor antagonist, diminished the anoxia-induced inhibition of CCDPK II activity in rat hippocampal and striatal slices. The significant protections were observed with Sch-23390 at 5 - 50 $\mu\text{mol} \cdot \text{L}^{-1}$ in rat hippocampal slices, whereas 20 - 100 $\mu\text{mol} \cdot \text{L}^{-1}$ in rat striatal slices, with the enzyme activity preserved up to 63.7 % and 66.1 % of the control, respectively (Fig 1).

Treatment with domperidone, a specific D_2 -like dopamine receptor antagonist, also produced a concentration-dependent attenuation of the inhibition of CCDPK II activity induced by anoxia in rat hippocampal and striatal slices. The significant protections were observed with domperidone at 0.01 $\mu\text{mol} \cdot \text{L}^{-1}$ in rat hippocampal slices, whereas 0.5 $\mu\text{mol} \cdot \text{L}^{-1}$ in rat striatal slice. The maximum protections were observed with domperidone at 1 $\mu\text{mol} \cdot \text{L}^{-1}$ in rat hippocampal slices, whereas 5 $\mu\text{mol} \cdot \text{L}^{-1}$ in rat striatal slice, the enzyme activity was preserved to 60.8 % and 56.5 % of the control, respectively (Fig 2).

DISCUSSION

Many mechanisms have been proposed to explain the dopaminergic neurotoxicity. One possibility is that direct dopamine receptor stimulation may mediate dopaminergic neurotoxicity. Dopamine receptors can be classified as "D₁-like" (D₁ and D₅) or "D₂-like" (D₂, D₃, and D₄) based upon both molecular biological and pharmacological similarities. D₁-like

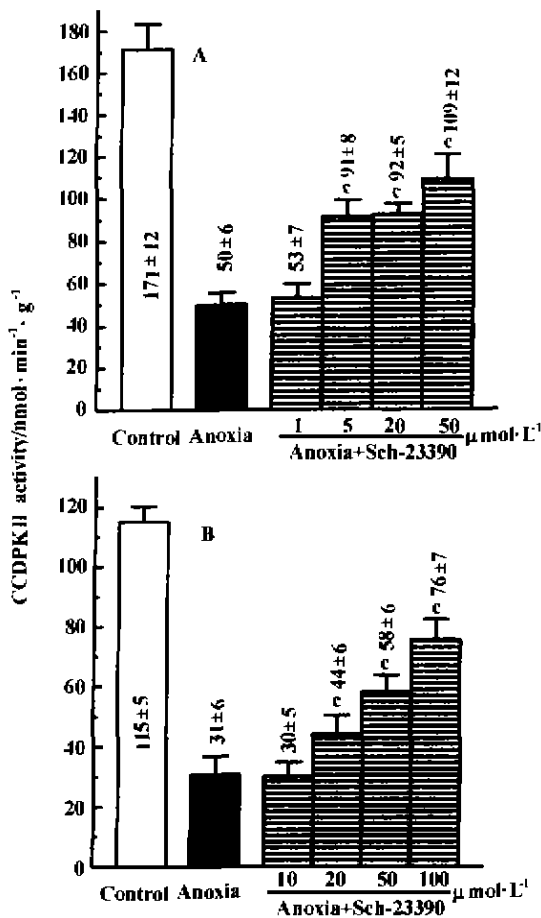


Fig 1. Concentration-dependent protection of Sch-23390 against anoxia-induced inhibition of CCDPK II activity in rat hippocampal (A) and striatal slices (B). $n=5$ rats. $\bar{x} \pm s$. $^cP < 0.01$ vs anoxia.

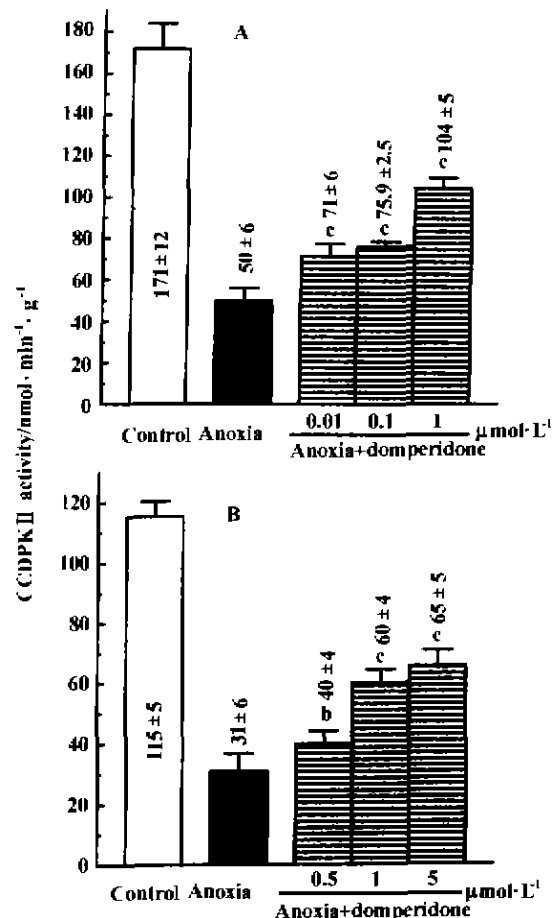


Fig 2. Concentration-dependent protection of domperidone against anoxia-induced inhibition of CCDPK II activity in rat hippocampal (A) and striatal slices (B). $n=5$ rats, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs anoxia.

and D₂-like receptors are present in the hippocampus and striatum. There have been conflicting reports about receptor subtypes mediating dopaminergic neurotoxicity during cerebral ischemia^[7,8].

The results of the present study showed that specific dopamine D₁-like or D₂-like receptor antagonist could diminish the inhibition of CCDPK II activity induced by anoxia in rat hippocampus and striatum. It suggests that the excessive release of dopamine during cerebral ischemia is involved in ischemia-induced inhibition of CCDPK II activity by stimulation of D₁-like or D₂-like receptors. Higher concentration was necessary in rat striatal slices for Sch-23390 or domperidone to cause significant protection, which might result from that more dopamine was released during cerebral ischemia or there were more dopamine receptors in the

striatum than those in the hippocampus^[4,9,10].

Calcium is a pivotal factor in ischemic neuronal damage. Many investigators have proved that D₁-like dopamine receptor mediated elevation of intracellular calcium levels by a variety of mechanisms. The relationship between D₂-like dopamine receptor and calcium levels varies with the host cells and tissues that have been studied^[4]. Our results suggests stimulation of D₂-like receptor mediates the inhibition of CCDPK II activity in rat hippocampus and striatum. The inhibition of CCDPK II activity is calcium-dependent. Therefore, our results may support the reports that stimulating D₂-like receptor subtypes can elevate the intracellular calcium concentration in rat hippocampus and striatum. Dopamine receptor antagonists could

only partially protect anoxia-induced inhibition of CCDPK II activity, which suggests that the inhibition of CCDPK II activity is also related with other factors, rather than dopamine receptors. This is consistent with our previous results which showed that glutamate receptors, voltage-gate calcium channel, etc were involved in the inhibition of CCDPK II activity during ischemia^[5,11]. The inhibition of CCDPK II activity is accompanied by the activation of Ca²⁺-calmodulin-independent form of the enzyme^[12]. The elevation of the intracellular calcium concentration results in an autophosphorylation of the enzyme at a threonine residue, Thr²⁰⁶. The autophosphorylation makes the kinase convert from the Ca²⁺-calmodulin dependent form to Ca²⁺-calmodulin-independent form, the Ca²⁺-calmodulin dependent activity is inhibited and the Ca²⁺-calmodulin independent activity is enhanced. Excessive activated CCDPK II can potentiate the presynaptic release of glutamate^[13], but also promote subsequent responsiveness of postsynaptic *N*-methyl-*D*-aspartate (NMDA) receptors or non-NMDA receptors to released glutamate by phosphorylating these receptors^[14,15], which results in enhancement of excitotoxicity of EAA. All above results may support our hypothesis that D₁-like and D₂-like receptors-stimulation partially contributes to dopaminergic neurotoxicity during cerebral ischemia by elevating intracellular calcium levels which leads to activation of calcium-dependent enzymes, including CCDPK II. On the other hand, the massive release of dopamine during cerebral ischemia could enhance excitotoxicity of glutamate by activation of CCDPK II.

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多巴胺受体拮抗剂对缺氧诱导的大鼠脑 Ca^{2+} -
钙调素依赖性蛋白激酶 II 活性抑制的保护作用¹

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关键词 多巴胺; 钙-钙调素依赖性蛋白激酶;
缺氧症; Sch-23390; 多潘立酮; 海马; 纹状体;
腺苷三磷酸

CCDPK II

目的: 研究多巴胺受体拮抗剂对缺氧诱导的大鼠
海马、纹状体脑片 Ca^{2+} -钙调素依赖性蛋白激酶 II
(CCDPK II) 活性抑制的影响。方法: 采用大鼠海

马、纹状体脑片体外缺氧模型(置于 95 % N_2 +
5 % CO_2)、以 ^{32}P -掺入法测定 CCDPK II 的活性。
结果: 缺氧 30 min、大鼠海马、纹状体脑片
CCDPK II 活性分别降低为对照的 29.2 % 和
27.0 %。Sch-23390 (特异性 D_1 -样多巴胺受体拮
抗剂) 和多潘立酮(特异性 D_2 -样多巴胺受体拮抗
剂) 对缺氧所诱导的酶活性抑制均可导致浓度依赖
的保护作用。CCDPK II 活性最大可恢复至对照
的 60 % 左右。结论: 多巴胺在缺氧诱导的大鼠海
马、纹状体 CCDPK II 活性抑制中有重要作用, 其
作用机制与 D_1 样和 D_2 样受体有关。

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