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Dopamine-glutamate interaction in rat striatal slices: changes of CCDPK Ⅱ, PKA, and LDH activity by receptor-mediated mechanisms[1]

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KEY WORDS dopamine; glutamates; dopamine receptors; *N*-methyl-*D*-aspartate receptors; corpus striatum; Ca^{2+}-calmodulin dependent protein kinase; cyclic AMP-dependent protein kinases; lactate dehydrogenase

ABSTRACT

AIM: To study the effects of dopamine (DA) and glutamate (Glu) and their receptor agonists/antagonists on Ca^{2+}/calmodulin-dependent protein kinase Ⅱ (CCDPK Ⅱ), cyclic AMP-dependent protein kinase A (PKA) activities and the LDH release in rat striatal slices, and to examine the interaction between DA and Glu transmitter systems in striatum. **METHODS**: The activities of CCDPK Ⅱ, PKA, and the release of LDH were determined with the ^{32}P-incorporation and colorimetry respectively in rat striatal slices. **RESULTS**: (1) Exogenous DA, D_1 receptor agonist SKF 38393 and D_2 receptor agonist LY 171555 reduced CCDPK Ⅱ activity in striatal slices; Glu also inhibited CCPPK Ⅱ activity in a concentration-dependent manner. NMDA receptor antagonist MK-801 could antagonize the inhibitory effect of SKF 38393 and LY 171555 on the CCDPK Ⅱ activity. D_1 and D_2 receptor antagonists SCH 23390 and spiperone could also antagonize the decrease of CCDPK Ⅱ activity induced by Glu; (2) DA and SKF 38393 markedly increased PKA activity in striatal slices, which was reduced by MK-801; (3) DA and Glu increased the release of LDH from the striatal neurons in a concentration-dependent manner. MK-801 antagonized the increase of LDH induced by DA. Spiperone, rather than SCH 23390, could reduce the release of LDH from striatal neuron in the presence of Glu. **CONCLUSION**: The interaction between DA and Glu transmitter systems is found in the regulation of the CCDPK Ⅱ and PKA activities and cell function in the striatum.

INTRODUCTION

The striatum plays a key role in the striatal- pallidal-thalamic feedback circuit that modulates the function of motor activity and cognitive abilities in the cerebral cortex. Striatal dysfunction can produce clinically important abnormalities of motor. Two most important afferent projections coming from cortex and substantia nigra to the striatum contain glutaminergic and dopaminergic fibers, respectively. Both glutamate (Glu) and dopamine (DA) extensively increased in the extracellular fluid during striatal ischemia[1]. Many evidences have shown that DA and Glu may interact *in vivo* to exacerbate neuronal damage in ischemia[2]. The excessive excitation of *N*-methyl-*D*-aspartate (NMDA) receptors is implicated in pathophysiology of ischemic stroke, hypoglycaemic brain damage and Huntington's disease. Glutamate receptor antagonists have also been shown to protect the striatum from hypoglycaemia and focal ischemia[3]. Moreover, the endogenous catecholamines are particularly involved in neuronal damage. It has been reported that lesioning the substantia nigra (SN) could protect the striatal neuron from damage in transient ischemia and excitotoxic insult[4].

Ca^{2+}/calmodulin dependent protein kinase Ⅱ (CCDPK Ⅱ) and cyclic AMP dependent protein kinase A (PKA) are both important regulating factors in the intracellular signal transduction. Their activities are sensitive to ischemia and are regulated by the alteration of intracellular free calcium and cAMP concentration. The changes of their activities may reflect a direct interference with

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dopamine-glutamate interaction at the receptor level. In addition, the leakage of lactic dehydrogenase (LDH) from neurons was considered as an indicator of the cell membrane damage.

Although the interaction between dopaminergic and glutaminergic pathways in striatum is well-recognized, the potential mechanisms underlying receptor-mediated neurotoxicity remain unclear. The effects of this interaction between DA and Glu on intracellular signal transduction, especially the changes of CCDPK Ⅱ and PKA activity have not been reported. The present experiments were specifically designed to determine that this interaction is mainly mediated by receptor-mediated mechanism. These experiments had two purposes. First, they determined whether or not the activities of CCDPK Ⅱ and PKA could be affected by DA, Glu and their interaction. Second, they examined the effect of this interaction on striatal lesion by measuring the release of LDH from striatal neurons.

MATERIALS AND METHODS

Chemicals Dopamine hydrochloride, (±)-SKF 38393 hydrochloride, R(+)-SCH 23390 hydrochloride, (−)quinpirole hydrochloride (LY 171555) and spiperone were from RBI, USA; (+)-5-methyl-10,11-dihydro-5H-dibenzo[a, d]cyclohepten-5, 10-imime maleate (MK-801), phenyl methyl sulphonyl fluoride (PMSF), glutamate (Glu), histone, sodium metabisulphite, and ATP were from Sigma, USA. [γ-^{32}P]ATP (185 PBq·mol^{-1}) was from Beijing Yahui Biomedical Co, China. Other reagents were of AR.

Rats Sprague-Dawley rats (♂, weighing 220 g ± s 20 g) were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade Ⅱ, Certificate № 005).

Striatal slices preparation and drug application Rats were decapitated and the brains were placed in ice-cold Krebs-Ringer solution (in mmol·L^{-1}: NaCl 122, KCl 3.1, KH_2PO_4 0.4, $MgSO_4$ 1.2, $CaCl_2$ 1.3, $NaHCO_3$ 25, glucose 10; pH 7.4) equilibrated with 95 % O_2 + 5 % CO_2. Striatal slices (350 μm) were prepared with a McILWAIN tissue chopper and preincubated in normal Krebs-Ringer solution at 37 ℃ for 90 min. DA receptor antagonists or MK-801 were added 10 min before exposure to Glu, DA, or DA receptor agonists. Sodium metabisulphite (200 μmol·L^{-1}) was added to the dopamine-containing solution to prevent the oxidation of dopamine. During preincubation and drug application, incubation medium was continuously bubbled with 95 % O_2 + 5 % CO_2. After experiments, the slices were quickly collected and frozen in liquid nitrogen.

Assay for CCDPK Ⅱ activity The slices were homogenized in a glass homogenizer on the ice bath with ice-cold homogenization buffer (in mmol·L^{-1}: Tris-HCl 20; egtazic acid 2, NaF 2, PMSF 0.5, mercaptoethanol (β-ME) 10; pH 7.5). The homogenate was spun at 10 000× g at 4 ℃ for 10 min, and the supernatant was assayed for CCDPK Ⅱ activity by the previous method[5]. The radioactivity was measured in a liquid scintillation spectrometer (LS-6500, Beckman). The CCDPK Ⅱ activity was expressed in terms of ^{32}P-incorporation.

Assay for PKA activity PKA activity was assayed at 30 ℃ for 5 min using histone as substrate in Tris/HCl buffer (20 mmol·L^{-1}, pH 7.4) containing $MgCl_2$ 5, 1,4-dithiothreitol (DTT) 5, egtazic acid 2, β-ME 10 mmol·L^{-1}, and p-nitrophenyl phosphate (PNPP) 10 g·L^{-1} with or without cAMP (0.5 μmol·L^{-1}). Phosphorylation reaction was started by addition of [γ-^{32}P]ATP (50 μmol·L^{-1}) and stopped after 5 min. The radioactivity was measured in a liquid scintillation spectrometer. The PKA activity was expressed in terms of ^{32}P-incorporation (nmol·min^{-1}·g^{-1} protein).

Assay for LDH activity The activity of LDH in the superfusate was measured using the colorimetry[6]. Enzyme activity was expressed as King's units of total protein content.

Determination of protein concentration Protein concentrations of samples were determined by the Lowry method with BSA as standard.

Statistical analysis Data were expressed as $\bar{x}$ ± s and analyzed by t-test.

RESULTS

Effect of MK-801 on CCDPK Ⅱ activity in rat striatal slices In the presence of DA, D_1 receptor agonist SKF 38393 or D_2 receptor agonist LY 171555, the CCDPK Ⅱ activities in striatal slices were decreased. NMDA receptor antagonist MK-801 antagonized the inhibitory effect of DA (10 μmol·L^{-1}), SKF 38393 (1 μmol·L^{-1}), and LY 171555 (1 μmol·L^{-1}). MK-801 increased concentration-dependently the CCDPK Ⅱ activity in the striatal slices in the presence of the drugs. MK-801 at 10 μmol·L^{-1} could obviously antagonize the DA,

SKF 38393 and LY 171555- induced inhibition of CCDPK Ⅱ activity (Tab 1).

Effect of MK-801 on PKA activity in rat striatal slices DA and D_1 agonist SKF 38393 increased PKA activity in rat striatal slices. In the presence of DA 10 $\mu mol \cdot L^{-1}$ and SKF 38393 1 $\mu mol \cdot L^{-1}$, the activity of PKA increased up to 167 % and 160 %, respectively. MK-801 reduced the increase of PKA activity induced by DA and SKF 38393 in a concentration-dependent manner (Tab 2).

Effect of SCH 23390 and spiperone on CCDPK Ⅱ activity in rat striatal slices Glu concentration-dependently decreased the activity of CCDPK Ⅱ in rat striatal slices. Glu 200 $\mu mol \cdot L^{-1}$ decreased the CCDPK Ⅱ activity in striatal slices from 84 ± 10 nmol $\cdot min^{-1} \cdot g^{-1}$ protein of the control to 34.7 ± 2.8 nmol $\cdot min^{-1} \cdot g^{-1}$ protein ($n = 6$, $P < 0.01$) in 30 min. D_1 receptor antagonist SCH 23390 and D_2 receptor antagonist LY 171555 could antagonize the effect of Glu on CCPPK Ⅱ activity. SCH 23390 20 $\mu mol \cdot L^{-1}$ and LY 171555 0.5 $\mu mol \cdot L^{-1}$ markedly increased CCDPK Ⅱ activity in the presence of Glu. However, the LY 171555 had more potent antagonism than SCH 23390 on inhibition of CCDPK Ⅱ activity induced by Glu (Tab 3).

Effect of MK-801 on LDH leakage from rat striatal slices DA could increase the leakage of LDH from the striatal slices owing to the damage of cell membrane. Along with the increase of DA concentration, the activity of LDH in superfusate was gradually increased (data not shown). MK-801 antagonized these increases of LDH activity induced by DA. The increase of LDH activity induced by DA was obviously reduced by MK-801 at concentration of 25 $\mu mol \cdot L^{-1}$ (Tab 4).

Effect of spiperone and SCH 23390 on the LDH leakage from striatal slices In the presence of Glu, the leakage of LDH from striatal neurons was increased in a concentration-dependent manner. Glu 100 $\mu mol \cdot L^{-1}$ increased the LDH activity in the superfusate

Tab 1. Effect of MK-801 on CCDPK Ⅱ activity ($nmol \cdot min^{-1} \cdot g^{-1}$ protein) in the presence of DA, SKF 38393 and LY 171555 in rat striatal slices. MK-801 was given 10 min before the addition of the drugs and the slices were incubated for a further 30 min. $n = 6$ rats. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^eP < 0.05$, $^fP < 0.01$ *vs* DA (10 μmol $\cdot L^{-1}$), $^gP > 0.05$, $^iP < 0.01$ *vs* SKF38393 (1 μmol $\cdot L^{-1}$); $^jP > 0.05$, $^lP < 0.01$ *vs* LY 171555 (1 μmol $\cdot L^{-1}$).

Treatment/ $\mu mol \cdot L^{-1}$	CCDPK Ⅱ activity	Treatment/ $\mu mol \cdot L^{-1}$	CCDPK Ⅱ activity	Treatment/ $\mu mol \cdot L^{-1}$	CCDPK Ⅱ activity
Control	84 ± 10	Control	84 ± 10	Control	84 ± 10
DA 10	34.5 ± 1.8^c	SKF 38393 1	39 ± 3.0^c	LY 171555 1	35.7 ± 2.5^c
MK-801 1 + DA 10	47 ± 5^{ce}	MK-801 1 + SKF 38393 1	39.7 ± 2.6^{cg}	MK-801 1 + LY 171555 1	38.2 ± 2.6^{cj}
MK-801 10 + DA 10	52 ± 3^{cf}	MK-801 10 + SKF38393 1	45.5 ± 2.5^{ci}	MK-801 10 + LY 171555 1	49 ± 7^{cl}
MK-801 25 + DA 10	54 ± 4^{cf}	MK-801 25 + SKF38393 1	51 ± 6^{ci}	MK-801 25 + LY 171555 1	53 ± 4^{cl}
MK-801 50 + DA 10	67 ± 5^{af}	MK-801 50 + SKF38393 1	61 ± 6^{bi}	MK-801 50 + LY 171555 1	64 ± 4^{bl}
MK-801 100 + DA 10	77 ± 3^{af}	MK-801 100 + SKF38393 1	70 ± 6^{ai}	MK-801 100 + LY 171555 1	76 ± 7^{al}

Tab 2. Effect of MK-801 on PKA activity in the presence of DA and SKF 38393 in the rat striatal slices. MK-801 was given 10 min before the addition of other drugs and the slices were incubated for a further 30 min. $n = 6$ rats. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* DA (10 μmol $\cdot L^{-1}$); $^iP < 0.01$ *vs* SKF38393 (1 μmol $\cdot L^{-1}$) group.

Treatment/ $\mu mol \cdot L^{-1}$	PKA activity $nmol \cdot min \cdot g^{-1}$ protein	Treatment/ $\mu mol \cdot L^{-1}$	PKA activity $nmol \cdot min \cdot g^{-1}$ protein
Control	130 ± 11	Control	130 ± 11
DA 10	217 ± 32^c	SKF 38393	208 ± 6^c
MK-801 1 + DA 10	162 ± 12^{cf}	MK-801 1 + SKF 38393 1	158 ± 6^{ci}
MK-801 10 + DA 10	140 ± 7^{bf}	MK-801 10 + SKF 38393 1	145 ± 4^{ci}
MK-801 25 + DA 10	133 ± 10^{af}	MK-801 25 + SKF 38393 1	129 ± 3^{ai}
MK-801 50 + DA 10	124 ± 17^{af}	MK-801 50 + SKF 38393 1	127 ± 4^{ai}
MK-801 100 + DA 10	150 ± 5^{cf}	MK-801 100 + SKF 38393 1	117 ± 5^{ai}

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Tab 3. Effect of SCH 23390 and spiperone on CCDPK Ⅱ activity in the presence of Glu in the rat striatal slices. SCH 23390 or spiperone was given 10 min before the addition of Glu for a further 30 min. $n = 6$ rats. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* Glu (200 μmol·L^{-1}) group.

Treatment/ μmol·L^{-1}	CCDPK Ⅱ activity nmol·min·g^{-1} protein	Treatment/ μmol·L^{-1}	CCDPK Ⅱ activity nmol·min·g^{-1} protein
Control	84 ± 10	Control	83 ± 10
Glu 200	34.7 ± 2.8^c	Glu 200	34.7 ± 2.8^c
SCH 23390 5 + Glu 200	40 ± 5^{cd}	Spiperone 0.1 + Glu 200	35.0 ± 2.5^{cd}
SCH 23390 10 + Glu 200	52 ± 4^{be}	Spiperone 0.5 + Glu 200	51 ± 3^{cf}
SCH 23390 20 + Glu 200	62 ± 4^{bf}	Spiperone 1.0 + Glu 200	63 ± 5^{af}
SCH 23390 50 + Glu 200	77 ± 4^{af}	Spiperone 5.0 + Glu 200	68 ± 5^{af}
SCH 23390 100 + Glu 200	75 ± 6^{af}	Spiperone 10 + Glu 200	76 ± 5^{af}

Tab 4. Effect of MK-801 on LDH activity in the presence of DA in the rat striatal slices. MK-801 was given 10 min before the addition of DA and the slices were incubated for a further 30 min. $n = 6$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control of MK-801 (−). $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* control of MK-801 (25 μmol·L^{-1}). $^gP > 0.05$, $^iP < 0.01$ *vs* MK-801 (−) group at the same concentration of DA.

Treatment/ μmol·L^{-1}	LDH activity (10^{-3} × king's unit·g^{-1} protein) MK-801 (−)	MK-801 (25 μmol·L^{-1})
Control	61 ± 5.4	58.4 ± 6.2
DA 10	72 ± 7.1^b	64.6 ± 5.3^{dg}
DA 20	89 ± 7.3^c	70.8 ± 8.1^{ci}
DA 50	113 ± 10.1^c	78.0 ± 7.4^{fi}

to 172 % of the control. D_2 antagonist spiperone at 5 μmol·L^{-1} could antagonize the increase of LDH activity induced by Glu. However, D_1 antagonist SCH 23390 had no obvious effect on the increase of LDH activity induced by Glu (Tab 5).

DISCUSSION

Dopaminergic and glutamatergic transmissions have long been known to interact at multiple level in the basal ganglia to modulate motor and cognitive functions[7]. Evidence has been accumulating that DA and Glu can interact both at pre-and postsynaptic levels in striatum. However, to our knowledge by now, there is no direct evidence verifying the effect of interaction between DA and Glu on CCDPK Ⅱ and PKA activities. Our results showed that the effects of DA and DA receptor agonists on the activities of CCDPK Ⅱ, PKA and LDH could be attenuated by NMDA receptor antagonist MK-801. DA receptor antagonist could also reduce the effects of Glu on these enzyme activities in striatal slices. In addition, MK-801 and spiperone had protective effect on striatal neuronal injury induced by DA and Glu, respectively. These results suggest that DA-Glu interaction could have a functional importance in modulating the Ca^{2+} and cAMP-dependent transmission in the striatum.

Both CCDPK Ⅱ and PKA are abundant in the striatum and play a crucial role in the cellular signal transduction. Their activities are regulated by intracellular concentration of Ca^{2+} and cAMP[8]. Glu and DA activate NMDA receptors and DA receptors and in turn increase intracellular second messages Ca^{2+} and cAMP[9],

Tab 5. Effect of spiperone and SCH 23390 on LDH activity in the presence of Glu in rat striatal slices. Spiperone or SCH 23390 was given 10 min before the addition of Glu and the slices were incubated for a further 30 min. $n = 6$ rats. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control. $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* no drug group at the same concentration of Glu.

Treatment/ μmol·L^{-1}	LDH activity (10^{-3} × king's unit·g^{-1} protein) No drug	Spiperone 5	Spiperone 10	SCH 23390 5	SCH 23390 10
Control	61 ± 6	61 ± 6	61 ± 4	60 ± 7	62 ± 4
Glu 10	72 ± 7^b	67 ± 8^{ad}	63 ± 6^{ae}	69 ± 5^{ad}	66 ± 7^{ad}
Glu 50	84 ± 9^c	74 ± 5^{ce}	65 ± 7^{af}	79 ± 5^{cd}	76 ± 10^{bd}
Glu 100	104 ± 13^c	82 ± 8^{cf}	72 ± 6^{cf}	94 ± 10^{cd}	89 ± 9^{ce}

respectively. The changes of CCDPK Ⅱ activity and PKA activity may reflect extracellular concentration of Glu and DA[10]. The leakage of LDH from striatal neurons can be used as an indicator of neuronal injury, especially membrane damage. Accumulating evidence suggests that both DA toxicity and Glu toxicity may be involved in the cell membrane damage and the leakage of LDH[11].

Our study revealed the decrease of CCDPK Ⅱ activity in the presence of DA, DA receptors agonists or Glu. This decrease of CCDPK Ⅱ activity measured *in vitro* reflexed the increase of CCDPK Ⅱ autophosphorylation *in vivo*. After autophosphorylation this kinase was converted from the Ca^{2+}/CaM-dependent form to Ca^{2+}/CaM-independent form, which results in the inhibition of Ca^{2+}/CaM-dependent activity. The DA receptor antagonists and MK-801 antagonized the inhibition of CCDPK Ⅱ activity induced by DA, DA receptor agonists and Glu, which reduce intracellular Ca^{2+} concentration mediated by presynaptic receptors and post-synaptic receptors[12].

Furthermore, under the experimental conditions, the increase of the PKA activity of striatal slices incubated with DA or D_1 agonist for a few minutes was attenuated by MK-801. This effect may be partially due to the inhibition of adenylate cyclase (AC) and activation of phosphodiesterase (PDE) by reducing cytosolic free Ca^{2+} and other unclear mechanisms[13].

As shown in the results, MK-801, a NMDA receptor antagonist, attenuated the effect of DA on LDH leakage from neurons, which indicates that MK-801 may partially protect striatal neurons against dopaminergic neurotoxicity. On another aspect, D_2 receptor antagonist spiperone had more potent protective effect than D_1 antagonist SCH 23390 on striatal neurons against Glu damage, suggesting that D_2 receptor-mediated Glu neurotoxicity plays more important role in striatal injury, which is consistent with some reports that the activation of D_2 receptor may increase intracellular Ca^{2+} concentration via mediating the function of NMDA receptor[2,14]. Based on the partial protective effect of spiperone and MK-801 on striatal neurons, the results suggest that the neurotoxicity of these neurotransmitters, such as oxidative stress and free radicals, might be also involved in the impairment of striatum[15].

In conclusion, the present work demonstrates that interactions between DA and Glu may influence different signal transduction pathways, CCDPK Ⅱ, and PKA activities. The findings suggest a possible detrimental interaction between DA and Glu. Further studies on the interaction mechanisms between DA and Glu may lead to a more detailed understanding of striatal neuronal injury by Glu and DA in cerebral ischemia, hypoxia, and other neuronal pathophysiological conditions.

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多巴胺和谷氨酸在纹状体脑片的相互作用：受体机制介导的 CCDPKⅡ，PKA 和 LDH 活性变化[1]

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关键词 多巴胺；谷氨酸盐类；多巴胺受体；*N*-甲基-*D*-天冬氨酸受体；纹状体；Ca^{2+}-钙调蛋白依赖性蛋白激酶；环一磷酸腺苷依赖性蛋白激酶类；乳酸脱氢酶

目的：研究 DA 和 Glu 及其受体激动剂/拮抗剂对大鼠纹状体脑片 Ca^{2+}/CaM 依赖性蛋白激酶Ⅱ(CCDPKⅡ)、cAMP 依赖性蛋白激酶(PKA)活性及乳酸脱氢酶(LDH)释放的影响，以探讨纹状体 DA 和 Glu 两个神经递质系统的相互作用. **方法**：用 ^{32}P 掺入法测定大鼠纹状体脑片 CCDPKⅡ 和 PKA 活性，用比色法测定 LDH 的释放. **结果**：(1) NMDA 受体拮抗剂 MK-801 能拮抗 DA、D_1 激动剂 SKF 38393 和 D_2 激动剂 LY 171555 对 CCDPKⅡ 活性的抑制作用. D_1 拮抗剂 SCH 23390 和 D_2拮抗剂 spiperone 均能拮抗 Glu 诱导的 CCDPKⅡ 活性降低. (2) DA 和 SKF 38393 显著增加纹状体脑片 PKA 活性，MK-801 可降低这种作用. (3) DA 和 Glu 增加 LDH 的释放并与浓度成正比. MK-801 拮抗 DA 诱导的 LDH 释放增加；spiperone 能显著减少 Glu 诱导的纹状体神经元 LDH 释放. **结论**：DA 和 Glu 的相互作用对调节纹状体神经元 CCDPKⅡ 和 PKA 活性及细胞功能是非常重要的.

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