

(-)-Stepholidine enhances K^+ depolarization-induced activation of synaptosomal tyrosine 3-monooxygenase from rat striatum¹

HU Gang^{2,3}, WU Yun-Ming³, JIN Guo-Zhang^{2,4}

(²Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031; ³Laboratory of Neuropharmacology, Xuzhou Medical College, Xuzhou 221002, China)

KEY WORDS depolarization; tyrosine 3-monooxygenase; protein kinases; dopamine receptors; stepholidine; corpus striatum; synaptosomes

AIM: To study the mechanism of K^+ depolarization-induced activation of synaptosomal tyrosine 3-monooxygenase (TM) in rat striatum and the effect of (-)-stepholidine (SPD) on this activation. **METHODS:** The TM was assayed for DOPA by HPLC-ECD; the activities of Ca^{2+} /calmodulin (CaM)-dependent protein kinase (PK II) and Ca^{2+} /phosphoinositide-dependent protein kinase (PKC) were assayed using histidine as substrate. **RESULTS:** The incubation of striatal synaptosomes in K^+ -riched ($60 \text{ mmol} \cdot \text{L}^{-1}$) medium resulted in a marked activation of TM. PKC inhibitor polymyxin B (PMB) completely blocked the activation of K^+ $60 \text{ mmol} \cdot \text{L}^{-1}$ on TM. Selective D_2 receptor agonist quinpirole (QP), Ca^{2+} removal from incubation medium and CaM antagonist W7 failed to affect the activation. However, SPD enhanced the activation of K^+ $60 \text{ mmol} \cdot \text{L}^{-1}$ on TM. Meanwhile, the incubation in K^+ $60 \text{ mmol} \cdot \text{L}^{-1}$ also activated PKC. Neither QP nor SPD affected K^+ depolarization-induced activation of PKC. **CONCLUSION:** The activation of K^+ depolarization on synaptosomal TM is enhanced by SPD and this activation is mediated by PKC rather than by PK II.

During the periods of increased impulse flow in central dopaminergic neurons the physiological properties of tyrosine 3-monooxygenase (TM) are altered, resulting in the activation of this rate-limiting enzyme in dopamine (DA) biosynthesis^(1,2). A similar activation can be produced *in*

vitro by K^+ ($60 \text{ mmol} \cdot \text{L}^{-1}$) depolarization of striatal slices or synaptosomes^(3,4). Ca^{2+} -dependent rather than cAMP-dependent phosphorylation events are responsible for TM activation due to K^+ depolarization^(4,5). However, it is still unclear that which Ca^{2+} -dependent protein kinase (PK II or PKC) is involved in this activation and whether such an activation is controlled by presynaptic DA autoreceptors. In the present study, we examined the mechanism which mediates the effect of K^+ depolarization on DA biosynthesis. The effect of SPD, a novel DA receptor antagonist⁽⁶⁾, on the activation of K^+ depolarization on TM was also investigated.

MATERIALS AND METHODS

Chemicals and reagents SPD ($[\alpha]_D -440^\circ$ in pyridine, mp = 161°C) was isolated from *Stephania intermedia* Lo; quinpirole hydrochloride (QP, Research Biochemicals Inc, USA); [γ -³²P]ATP, Radio-Medicine Institute, China); histidine, phorbol 12, 13-dibutyrate (PDB), polymyxin B (PMB), N-6-aminohexyl-5-chloro-1-naphthalenesulfonamide (W7), HEPES, DOPA, D, L-6-methyl-5, 6, 7, 8-tetrahydropterin dihydrochloride (6-MPH₄), and catalase (Sigma); β -mercaptoethanol (Shanghai Fourth Reagent Factory); L-tyrosine (Shanghai Institute of Biochemistry); 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015, Aldrich Chemie, Germany); D-camphor-10-sulphonic acid (CSA, BDH Chemicals Ltd, England). All other chemicals were AR.

Rats Sprague-Dawley rats ($231 \pm 17 \text{ g}$, Shanghai Laboratorial Animal Center) were used.

Preparation of striatal synaptosomes After decapitation of rat, the striatum were taken into ice-cold homogenizing medium (sucrose 320, β -mercaptoethanol 2, and Tris-HCl 20 $\text{mmol} \cdot \text{L}^{-1}$, pH 7.45). Each preparation was pooled from 10 rats. And then synaptosomes were prepared⁽⁷⁾.

TM assay TM activity was assayed for DOPA by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Enzymatic reaction was proceeded⁽⁸⁾ after synaptosomes were preincubated at 37°C for 10 min with HEPES 50 $\text{mmol} \cdot \text{L}^{-1}$ buffer (pH 7.3)

¹Supported by the National Natural Science Foundation of China, No 39130091 and No 39300162

⁴Correspondence to Prof JIN Guo-Zhang.

Received 1995-07-11

Accepted 1996-05-24

containing NaCl 128, KCl 4.8, CaCl₂ 1.3, MgSO₄ 1.2, Na₂HPO₄ 15.8, and glucose 10 mmol · L⁻¹. When incubation was performed in K⁺-enriched medium (K⁺ 60 mmol · L⁻¹), the concentration of NaCl was reduced to 72.8 mmol · L⁻¹ to maintain a constant osmolarity^[3]

HPLC-ECD The HPLC-ECD system consisted of Model 510 pump (Waters), a Model U6K Universal injector (Waters), and two reverse-phase Linchrosob C₁₈ columns (50 mm · 4 mm, 200 mm · 4 mm, 5 μm). The column elute was monitored with a Waters 460 Electrochemical Detector (ECD) at 0.7 V. The mobile phase was a degassed mixture of ClCH₂COOH 0.16 mol · L⁻¹, NaOH 0.1 mol · L⁻¹, edetic acid 0.1 mmol · L⁻¹, CSA 25 mmol · L⁻¹ and methanol 10 % (pH 2.8). The flow rate was 1 mL · min⁻¹

Assay of synaptosomal protein kinases The activities of Ca²⁺/CaM-dependent protein kinase (PK II) and Ca²⁺/phosphoinositide-dependent protein kinase (PKC) were assayed using histidine as substrate^[9]. The activity of protein kinases were expressed with the amounts of P, incorporated into histidine from [³²P]ATP (nmol · g⁻¹ · min⁻¹).

Protein assay Protein of synaptosomes was determined^[10] and bovine serum albumin was used as standard.

Statistical analysis of data All data were analyzed with *t*-test.

RESULTS

Effects of Ca²⁺ removal and W7 on activity of synaptosomal TM during K⁺ depolarization In the presence of K⁺ 60 mmol · L⁻¹, the activity of synaptosomal TM from rat striatum was markedly increased (*P* < 0.01). When Ca²⁺ was removed from the incubation medium, there was no significant change in K⁺-activated TM activity. CaM inhibitor W7 failed to affect the activation of K⁺ 60 mmol · L⁻¹ on the activity of synaptosomal TM (Tab 1).

PMB against activation of K⁺ and PDB on activity of synaptosomal TM When the PKC activator PDB (1 μmol · L⁻¹) was incubated with striatal synaptosomes, the activity of TM was increased by 43 % (*P* < 0.01). This activation of synaptosomal TM was completely blocked by selective PKC inhibitor PMB 10 μmol · L⁻¹. PMB also abolished the activation of K⁺ 60 mmol · L⁻¹ on the activity of synaptosomal TM from rat striatum (Tab 1).

Effects of SPD and QP on activation of PDB on activity of synaptosomal TM Specific PKC

Tab 1. Effects of W7, PMB, and Ca²⁺ removal on K⁺ depolarization-induced activation of synaptosomal tyrosine 3-monoxygenase from rat striatum. *n* = 4 homogenates (each was pooled from 10 rats and assayed in triplicate). **P* > 0.05, ^c*P* < 0.01 vs control; ^d*P* > 0.05, ^f*P* < 0.01 vs PDB or K⁺ 60 mmol · L⁻¹.

Groups	TM activity/DOPA nmol · g ⁻¹ · min ⁻¹
Control (Ca ²⁺ in medium)	254 ± 47
K ⁺ 60 mmol · L ⁻¹	459 ± 81 ^c
K ⁺ 60 mmol · L ⁻¹ + Ca ²⁺ removal	442 ± 84 ^{cd}
K ⁺ 60 mmol · L ⁻¹ + W7	438 ± 76 ^{cd}
Control	243 ± 33
PDB 1 μmol · L ⁻¹	335 ± 38 ^c
PDB 1 μmol · L ⁻¹ + PMB 10 μmol · L ⁻¹	254 ± 41 ^{df}
K ⁺ 60 mmol · L ⁻¹	432 ± 29 ^c
K ⁺ 60 mmol · L ⁻¹ + PMB 10 μmol · L ⁻¹	248 ± 57 ^{df}

activator PDB increased the activity of synaptosomal TM from rat striatum and the formation of levodopa in a concentration-dependent manner. TM activities were increased by 30 %, 50 %, and 74 % at PDB 1, 10, and 100 μmol · L⁻¹, respectively. Neither SPD nor QP affected the activation of PDB on the activity of synaptosomal TM (Tab 2).

Tab 2. Effects of SPD and QP on activation PDB on activity of synaptosomal tyrosine 3-monoxygenase from rat striatum. *n* = 4 homogenates (each was pooled from 10 rats and assayed in triplicate). Control activity was 220 ± 40 DOPA nmol · g⁻¹ · min⁻¹. ^b*P* < 0.05, ^c*P* < 0.01 vs control.

PDB/ μmol · L ⁻¹	TM activity/DOPA nmol · g ⁻¹ · min ⁻¹		
	PDB alone	QP/10 μmol · L ⁻¹	SPD/100 μmol · L ⁻¹
0.01	238 ± 40	231 ± 54	233 ± 53
0.1	286 ± 43	276 ± 37	280 ± 77
1	310 ± 30 ^b	306 ± 33 ^b	301 ± 27 ^b
10	356 ± 51 ^c	349 ± 61 ^c	343 ± 42 ^c
100	415 ± 74 ^c	406 ± 71 ^c	422 ± 69 ^c

SPD enhancing K⁺ activation on activity of synaptosomal TM Under the "normal" condition (K⁺ 4.8 mmol · L⁻¹), the activity of synaptosomal TM was 261 ± 60 levodopa nmol · g⁻¹ · min⁻¹. When the K⁺ concentration in incubating medium was increased to 60 mmol · L⁻¹, the activity of synaptosomal TM was increased 79 % (*P* < 0.01). Selective D₂ receptor agonist QP (0.01 - 100

$\mu\text{mol}\cdot\text{L}^{-1}$) did not affect the activation of K^+ $60\text{ mmol}\cdot\text{L}^{-1}$ on TM activity. However, SPD enhanced the activation of K^+ $60\text{ mmol}\cdot\text{L}^{-1}$ on the activity of synaptosomal TM at $>1\ \mu\text{mol}\cdot\text{L}^{-1}$ (Tab 3).

Tab 3. Effects of SPD and QP on K^+ depolarization-induced activation of synaptosomal tyrosine 3-monoxygenase from rat striatum. $n = 4$ homogenates (each was pooled from 10 rats and assayed in triplicate). Basal activity was $261 \pm 60\ \text{nmol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$. ^b $P < 0.05$. ^c $P < 0.01$ vs K^+ -activated activity (0).

Concentrations/ $\mu\text{mol}\cdot\text{L}^{-1}$	TM activity/DOPA QP	$\text{nmol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ SPD
0	467 ± 57	467 ± 57
0.01	462 ± 53	476 ± 73
0.1	459 ± 76	479 ± 81
1	457 ± 39	653 ± 32 ^b
10	448 ± 62	681 ± 48 ^c
100	443 ± 67	695 ± 53 ^c

Effects of SPD and QP on activities of synaptosomal PK II and PKC In the presence of K^+ $60\text{ mmol}\cdot\text{L}^{-1}$, the activity of PKC was increased ($P < 0.05$). However, the PK II activity was not changed significantly. Both SPD and QP failed to affect the activation of K^+ $60\text{ mmol}\cdot\text{L}^{-1}$ on synaptosomal PKC (Tab 4).

Tab 4. Effects of SPD and QP on activities of synaptosomal PK II and PKC from rat striatum. $n = 4$ homogenates (each was pooled from 10 rats and assayed in triplicate). ^b $P < 0.05$ vs control; ^d $P > 0.05$ vs K^+ $60\text{ mmol}\cdot\text{L}^{-1}$.

Groups	PK II activity/ $\text{P}_i\ \text{nmol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$	PKC activity/ $\text{P}_i\ \text{nmol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$
Control	563 ± 41	253 ± 56
K^+ $60\text{ mmol}\cdot\text{L}^{-1}$	585 ± 73	387 ± 21 ^b
K^+ $60\text{ mmol}\cdot\text{L}^{-1}$ + QP/ $\mu\text{mol}\cdot\text{L}^{-1}$		
10	583 ± 64	377 ± 14 ^{bd}
100	573 ± 82	360 ± 26 ^{bd}
K^+ $60\text{ mmol}\cdot\text{L}^{-1}$ + SPD/ $\mu\text{mol}\cdot\text{L}^{-1}$		
10	567 ± 57	376 ± 43 ^{bd}
100	571 ± 94	374 ± 62 ^{bd}

DISCUSSION

The results in the present study confirm that K^+ depolarization stimulates the activity of synaptosomal TM and increases DA biosynthesis.

The stimulatory effects of K^+ depolarization on TM were not affected by both Ca^{2+} removal from incubating medium and CaM antagonist W7. These results indicate that the increase of DA biosynthesis is not mediated by the phosphorylation of Ca^{2+} /CaM-dependent PK II on TM. On the other hand, selective PKC inhibitor PMB could completely block the activations of both K^+ and PDB on synaptosomal TM. Meanwhile, synaptosomal PKC but not PK II was markedly activated by the incubation of K^+ $60\text{ mmol}\cdot\text{L}^{-1}$. These results show that the activation of K^+ depolarization on TM is mediated by the phosphorylation of Ca^{2+} /phosphoinositide-dependent PKC on TM.

Selective D_2 receptor agonist QP failed to affect the activation of K^+ on synaptosomal TM, suggesting that the K^+ depolarization-induced increase of DA biosynthesis is not regulated by presynaptic D_2 autoreceptors. This is strongly supported by the results that QP couldn't affect the activation of PDB, a specific PKC activator, on the activity of synaptosomal TM and that QP couldn't affect the activation of K^+ $60\text{ mmol}\cdot\text{L}^{-1}$ on PKC.

SPD is a novel DA receptor antagonist which can reverse the negative feedback regulation of presynaptic DA autoreceptors on DA biosynthesis⁽⁸⁾. The results in the present study show that SPD enhanced the activation of K^+ depolarization on synaptosomal TM whereas it was ineffective to TM activity under normal condition (K^+ $4.8\text{ mmol}\cdot\text{L}^{-1}$). This enhanced effect can be explained by the proposal that endogenous DA released in the synaptic cleft inhibits TM activity *via* the stimulation of functional presynaptic autoreceptors on striatal dopaminergic terminals⁽¹¹⁾ and that SPD can block this inhibitory effect of endogenous DA *via* blocking presynaptic DA autoreceptors^(6,8,11).

REFERENCES

- 1 El Mostikawy S, Glowinski J, Hamon M. Tyrosine hydroxylase activation in depolarized dopaminergic terminals-involvement of Ca^{2+} -dependent phosphorylation. *J Neurochem* 1985; 45: 173-84.
- 2 Togari A, Kato T, Nagatsu T. Studies on the regulatory mechanism of tyrosine hydroxylase system in adrenal slices by using high-performance liquid

chromatography with electrochemical detection.
 Biochem Pharmacol 1982; 31: 1729 - 34.

3 El Mestigawy S, Glowinski J, Hamon M. 49~52
 Presynaptic dopamine autoreceptors control tyrosine hydroxylase
 activation in depolarized striatal dopaminergic terminals.
 J Neurochem 1986; 46: 12 - 22.

4 Zigmond RE, Schwarzschild MA, Rittenhouse AR.
 Acute regulation of tyrosine hydroxylase by nerve activity and by
 neurotransmitters via phosphorylation.
 Annu Rev Neurosci 1989; 12: 415 - 61.

5 Goldstein M, Greene LA. Activation of tyrosine hydroxylase by
 phosphorylation. In: Meltzer RE, editor. Psychopharmacology:
 the third generation of progress
 New York: Raven, 1987; 75 - 80.

6 Jin GZ. (-)-Tetrahydropalmatine and its analogues as new
 dopamine receptor antagonists.
 Trends Pharmacol Sci 1987; 8: 81 - 2.

7 Hu G, Hu Y, Jin GZ. Effects of *l*-stepholidine on synaptosomal
 Ca²⁺-ATPase and subcellular calmodulin in rat striatum.
 Acta Pharmacol Sin 1992; 13: 307 - 11.

8 Hu G, Jin GZ. (-)-Stepholidine antagonizes the inhibition by
 D₂ receptor agonists on synaptosomal tyrosine hydroxylase in rat
 corpus striatum. Acta Pharmacol Sin 1995; 16: 376 - 9.

9 Hashimoto Y, Soderling TR. Calcium · calmodulin-dependent
 protein kinase II and calcium · phospholipid-dependent protein
 kinase activities in rat tissues assayed with a synthetic peptide.
 Arch Biochem Biophys 1987; 252: 418 - 25.

10 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ.
 Protein-measurement with the Folin phenol reagent.
 J Biol Chem 1951; 193: 265 - 75.

11 Onali P, Mosca E, Olanas MC.
 Presynaptic dopamine autoreceptors and second messengers
 controlling tyrosine hydroxylase activity in rat brain.

Neurochem Int 1992; 20 Suppl: 89C - 93C.

左旋千金藤立定增强 K⁺ 去极化对大鼠纹状体酪氨酸 3-单加氧酶的激活作用¹

胡刚^{2,3}, 吴云明³, 金国章^{2,4}
 (中国科学院上海药物研究所, 上海 200031; ³徐州医学院神经药理研究室, 徐州 221002, 中国)

关键词 去极化; 酪氨酸 3-单加氧酶; 蛋白激酶类; 多巴胺受体; 千金藤立定; 纹状体; 突触体

目的: 研究 K⁺ 去极化对大鼠纹状体突触体酪氨酸 3-单加氧酶(TM)激活的机制及 SPD 对此激活的影响. 方法: 应用 HPLC-ECD 法测定 DOPA 为 TM 的活性, 并应用同位素法测定 PK II 和 PKC 的活性. 结果: SPD 1, 10 和 100 μmol·L⁻¹ 增强 K⁺ 去极化对突触体 TM 的活性, PKC 抑制剂 PMB 10 μmol·L⁻¹ 能完全逆转 K⁺ 去极化的激活效应, 而选择性 D₂ 受体激动剂 QP, CaM 拮抗剂 W7 和去除反应液中的 Ca²⁺ 均不影响 K⁺ 去极化对 TM 的激活. K⁺ 去极化使突触体 PKC 的活性增加 53 %, 而对 PK II 的活性无影响. SPD 和 QP 均不影响 K⁺ 去极化对 PKC 的激活. 结论: K⁺ 去极化对突触体 TM 的激活作用是由 PKC 介导的, 不受突触前 DA 自身受体的调控, 但被 SPD 增强.

Information for authors

Acta Pharmacol Sin 1997 Jan; 18 (1); I - VI
 Br Med J 1991 Feb 9; 301 (6772); 338 - 41
 N Engl J Med 1991 Feb 7; 324 (6): 424 - 8

Reprints of any article published in Acta Pharmacologica Sinica may be purchased from the Institute for Scientific Information, 3501 Market Street, Philadelphia PA 19104, USA.

Fax: 1-215-386-6362. Phone: 1-215-386-0100.