

差示光谱 $\lambda_{max} = 385 \text{ nm}$, $\lambda_{min} = 420 \text{ nm}$. II 型差示光谱 $\lambda_{max} = 425 \sim 435 \text{ nm}$, $\lambda_{min} = 390 \sim 410 \text{ nm}$. VX 与 P-450 结合显示典型的 I 型差示光谱, 苯胺与 P-450 结合显示 II 型差示光谱. 苯胺对微粒体悬液以 VX 为底物的酶促反应产生明显的抑制作用. I 型化合物通常是 MFO 的底物, II 型化合物通常是 MFO 以 I 型化合物为底物时的竞争性抑制剂⁽⁷⁾. 综合以上结果证明 VX 是 MFO 的底物, 即肝脏中催化 VX 需氧代谢的酶是微粒体中的 MFO.

REFERENCES

- 1 Strobel HW, Lu AYH, Heidema J, Coon MJ. Phosphatidylcholine requirement in the enzymatic reduction of hemoprotein P-450 and in fatty acid, hydrocarbon, and drug hydroxylation. *J Biol Chem* 1970; 245 : 4851.
- 2 Fu FH, Sun MC. Occurrence of VX oxidase. *Chin J Pharmacol Toxicol* 1989; 3 : 264
- 3 Coon MJ. Reconstruction of cytochrome P-450-containing mixed-function oxidase system of liver microsomes. *Methods Enzymol. Biomembrances Part C* 1978; 52 : 200.
- 4 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951; 193 : 265.
- 5 Imai Y, Ito A, Sato R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. *J Biochem* 1966; 60 : 417.
- 6 Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes, I. Evidence for its hemoprotein nature. *J Biol Chem* 1964; 239 : 2370.
- 7 Jefcoate CR. Measurement of substrate and inhibitor binding to microsomal cytochrome P-450 by optical-difference spectroscopy. *Methods Enzymol. Biomembrances Part C* 1978; 52 : 258.

中国药理学报 *Acta Pharmacologica Sinica* 1990 Mar, 11 (2) : 126-130

环胍苯砒对乙酰胆碱酯酶的抑制性质

袁伯俊¹、秦伯益 (军事医学科学院毒物药物研究所, 北京 100850, 中国)

Inhibitory properties of cycloguanide phenylsulfone on acetylcholinesterase

Yuan Bo-Jun, Qin Bo-Yi
(Institute of Pharmacology & Toxicology, Academy of Military Medical Sciences, Beijing 100850, China)

ABSTRACT Mouse brain homogenates, mouse RBC, immobilized enzyme of pig brain, and human RBC were chosen as source of AChE. AChE activities were determined by colorimetric and gasometric methods. Cycloguanide phenylsulfone (CGP) exerted a moderate inhibitory effect on AChE. The pl_{50} (negative logarithm of molar concentration causing 50% inhibition of AChE) towards AChE in mouse RBC and brain were

5.75 and 5.50, respectively. The binding potency to AChE was very loose. The AChE inhibition was easily reversed by washing. It showed that CGP belonged to the contra-competitive AChE inhibitor.

KEY WORDS acetylcholinesterase; cholinesterase inhibitors; cycloguanide phenylsulfone; sulfones; triazines; physostigmine; soman

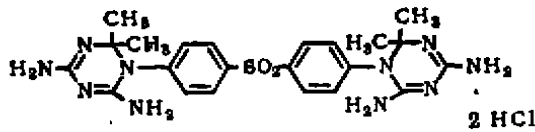
摘要 环胍苯砒对真性胆碱酯酶(小鼠全脑、小鼠 RBC、人 RBC AChE 和猪脑固相酶)有选择性抑制作用, 结合疏松、抑制酶易恢复, 是 AChE 的一种反竞争性抑制剂。

关键词 乙酰胆碱酯酶; 乙酰胆碱酯酶抑制剂; 环胍苯砒; 砒类; 三嗪; 毒扁豆碱; 索曼

Received 1988 Nov 18 Accepted 1989 Aug 29
¹ Now in Laboratory for Antimalarial Drug Research, Second Military Medical University of PLA, Shanghai 200433, China

环胍苯砒 (cycloguanide phenylsulfone, CGP) 化学名为双-对-(4,6-二氨基-2,2-二甲基-1,2-二氢均三嗪)二苯砒盐酸盐(1,1-

(sulfonyl di-*p*-phenylene)bis-(4,6-diamino-2,2-dimethyl-1,2-dihydro-*s*-triazine) dihydrochlorides)。国外最早曾作为化疗药筛选过⁽¹⁾，没有报道过它的药理作用。本所宋鸿鏞等1972年在过筛时发现环胍苯砒对神经性毒剂在小鼠上有一定的预防作用。闻思真等1975年证实环胍苯砒对ChE有较弱的抑制作用和保护作用，但不同于毒扁豆碱类酶保护药，没有重活化作用(未发表资料)。本文观察它对真性胆碱酯酶(AChE)的作用，以探讨抗神经性毒剂的新类型药物。



Cycloguanide phenylsulfone

MATERIALS AND METHODS

药物 环胍苯砒、碘化硫代乙酰胆碱(acetylthiocholine iodide)和索曼(soman)由本所提供,毒扁豆碱(physostigmine),英国进口。

酶源 小鼠全脑匀浆和血球AChE、猪脑固相AChE和人红血球AChE(人血采自本所自愿献血者)为真性胆碱酯酶(true ChE or AChE)。血浆ChE为假性ChE(pseudo-ChE Or BuChE)。小鼠全血ChE中, AChE: BuChE为1:13,因此主要反映假性胆碱酯酶活性。

ChE活力测定方法系采用比色法^(2,3),或气体定量法⁽⁴⁾。未说明测定方法者均为比色法。

抑制剂和AChE结合的可逆性试验 体外试验用抑制90%酶活力的抑制剂浓度(I_{90})和小鼠血球AChE或猪脑固相酶一起孵温15 min后,离心洗涤或抽滤洗涤多次,观察酶活性的恢复。用一次低温高速离心(0℃, 20 000 × g, 30 min)和两次常温离心(30℃, 2 000 × g, 10 min)分离血球和血浆,观察离心的温度对体内抑制酶活力测定的影响。

对小鼠全脑匀浆AChE抑制类型试验⁽⁵⁾

Tab 1. Negative log mol/L causing 50% inhibition of AChE (pI_{50}) of cycloguanide phenylsulfone, physostigmine and soman towards ChE in mouse blood, plasma, RBC and brain *in vitro*. Inhibition rates of blood and plasma ChE by 0.1 mmol cycloguanide phenylsulfone were 28.45 and 9.61%, respectively. $n=3$, $\bar{x} \pm SD$.

	Cycloguanide phenylsulfone	Physostigmine	Soman
Blood	—	7.30 ± 0.11	8.30 ± 0.07
Plasma	—	7.37 ± 0.11	7.40 ± 0.09
RBC	5.75 ± 0.15	6.75 ± 0.13	9.00 ± 0.13
Brain	5.50 ± 0.15	6.30 ± 0.10	8.90 ± 0.13

1:9小鼠全脑匀浆AChE 0.1 ml和底物(5 mmol 硫代乙酰胆碱)及抑制剂同时孵温(37.5℃) 3 min,用比色法测酶活性。观察在抑制剂存在下,底物浓度对反应速度的影响。采用Lineweaver-Burk方法作图,判断抑制类型。

RESULTS

对AChE的选择性抑制 环胍苯砒在预防神经性毒剂中毒时,对小鼠血球AChE的保护作用明显高于血浆ChE。表明环胍苯砒在小鼠体内对ChE的抑制可能有选择性,为此用体外试验方法观察环胍苯砒对胆碱酯酶的抑制作用,结果证实环胍苯砒对真性胆碱酯酶有选择性抑制作用,可以测出 pI_{50} (Tab 1)而对全血ChE(0.1 mmol,抑制率为28.45%)和血浆ChE(0.1 mmol,抑制率为9.61%)则测不出 pI_{50} 。同时,用gasometric法测环胍苯砒抑制小鼠全脑匀浆AChE的 pI_{50} 为5.3,用比色法测环胍苯砒抑制猪脑固相酶的 pI_{50} 为5.3,抑制人红血球AChE的 pI_{50} 为5.25。说明用不同方法和不同酶源,均能测出环胍苯砒对AChE的抑制率 pI_{50} ,且 pI_{50} 水平基本一致。可以说,环胍苯砒对真性胆碱酯酶有选择性抑制作用。

在体内试验时,开始用常法(30℃, 2 000 × g, 10 min,离心洗涤二次)没有测出环胍苯砒对小鼠血球AChE的抑制,可能在离心

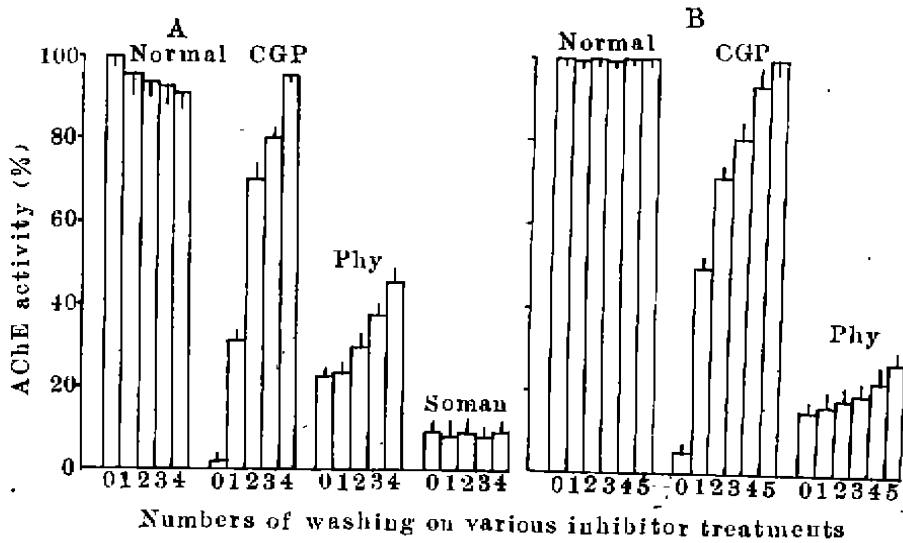


Fig 1. (A) Stability of RBC AChE in mouse after cycloguanide phenylsulfone (CGP, 0.5 mmol/L), physostigmine (Phy, 10 μ mol/L) and soman 10 nmol/L by centrifugalization washing *in vitro*. 2000 \times g, 30 $^{\circ}$ C, 10 min 2 times. (B) Stability of modified AChE in pig brain after CGP and Phy by washing. n = 4, $\bar{x} \pm SD$.

洗涤时，环胍苯砒抑制酶又恢复了活性。所以采用一次低温高速离心(0 $^{\circ}$ C, 20 000 \times g, 30 min)就测出了环胍苯砒对小鼠血球 AChE 的抑制率为 37%，对小鼠血浆 ChE 的抑制率为 5% (ip LD₅₀/4 环胍苯砒 15 min 后活杀)。而等毒性剂量(ip LD₅₀/4 15 min 后活杀)的毒扁豆碱同样条件下离心，对小鼠血球 AChE 和血浆 ChE 的抑制率分别为 30 和 29%。说明和毒扁豆碱不同，环胍苯砒对真性胆碱酯酶有选择性抑制作用。总之，体内外试验均表明环胍苯砒对真性胆碱酯酶的选择性抑制作用。

和 AChE 的可逆性结合 如前所述，用常法测不出环胍苯砒体内给药对血球 AChE 的抑制，可能是在离心洗涤分离血球时环胍苯砒已从酶分子上迅速解离的缘故。为此观察了体外用环胍苯砒抑制血球 AChE，然后用不同离心洗涤条件再测酶活性，并和毒扁豆碱及索曼作比较，结果表明，随着离心洗涤次数的增加，正常酶有小部分丢失，酶活力下降约 10%；环胍苯砒抑制酶经 4 次离心洗涤，其活性几乎全部恢复；毒扁豆碱抑制酶活力也有部分恢复，

索曼抑制酶活力没有什么变化 (Fig 1)。可见，环胍苯砒和血球 AChE 结合很疏松，解离较快。

环胍苯砒抑制猪脑固相酶的稳定性试验也表明其和猪脑固相酶结合疏松，解离很快。而

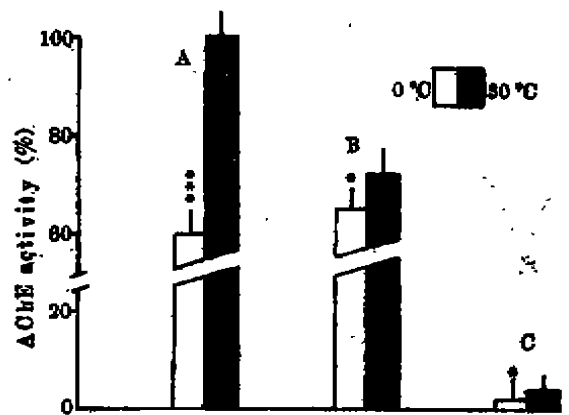


Fig 2. Stability of mouse RBC AChE after ip LD₅₀/4. A) CGP 5 mg/kg, B) Phy 0.25 mg/kg and C) soman 90 μ g/kg by centrifugalization washing at different temperatures. n = 4, $\bar{x} \pm SD$, *P > 0.05, ***P < 0.01.

毒扁豆碱则结合较紧密,解离较慢(Fig 2 A)。

离心的温度对测定环胍苯砒抑制小鼠血球 AChE 活力影响显著,对毒扁豆碱也有些影响,而对索曼则没有明显影响(Fig 2 B)。这也说明环胍苯砒和 AChE 结合的稳定性差,离心的温度增加也促使环胍苯砒和 AChE 形成的复合物解离。

对 AChE 的反竞争性抑制

环胍苯砒对 AChE 的作用不同于毒扁豆碱和索曼。故进而观察其对 AChE 的抑制动力学,并和毒扁豆碱及索曼作比较。结果表明,索曼抑制小鼠全脑匀浆 AChE 的动力学是典型的竞争型抑制图形。AChE 抑制剂动力学研究表明,有机磷酸酯类(DFP, paraoxon, sarin, soman 等)是 AChE 的不可逆竞争性抑制剂^(11,12);毒扁豆碱为非竞争型,这和文献⁽³⁾结果相符;而环胍苯砒为反竞争型抑制图型(Fig 3)。

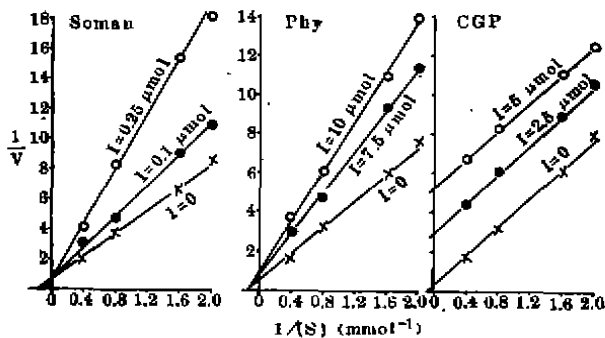


Fig 3. Double-reciprocal plots of AChE in mouse brain after soman, Phy and CGP. AChE was incubated with acetylthiocholine iodide (5 mmol) and 0.1 mol phosphate buffer (pH 7.4) for 3 min at 37.5°C.

DISCUSSION

环胍苯砒对小鼠全脑、小鼠 RBC、人 RBC 和猪脑固相 AChE(真性胆碱酯酶)均有抑制作用, pI_{50} 为 5.25-5.75。而对小鼠全血(90%是假性 ChE)和血浆 ChE(假性 ChE)则

测不出 pI_{50} 。体内试验也表明环胍苯砒对真性 ChE 的明显抑制(40%),而对假性 ChE 则几乎没有抑制(抑制率<5%),可以认为环胍苯砒对真性 ChE 有选择性抑制作用。近年来,真性 ChE 抑制剂的研究也有报道,石杉碱甲⁽⁹⁾和催醒宁⁽¹⁰⁾就是真性 ChE 抑制剂,并已试用临床。环胍苯砒能否用于临床有待深入研究。

环胍苯砒和 AChE 结合疏松,离心洗涤多次即可使酶活性恢复。这可能是因为生理 pH 条件下,环胍苯砒分子环胍基上的氮原子质子化而显正电性,就可能和 AChE 的外周阴离子部位呈遮蔽性结合,起一种桥式封闭作用。这种结合当然没有象毒扁豆碱和索曼与 AChE 酯解部位呈共价键结合来得牢固。假性 ChE 没有阴离子部位⁽⁶⁾,所以环胍苯砒对其作用很弱。我们也观察环胍苯砒对 α -糜蛋白酶活力的影响,结果环胍苯砒 5 mmol 反抑制 10%,作用也很弱,而 α -糜蛋白酶也是没有阴离子部位的。

环胍苯砒对 AChE 呈反竞争型抑制,说明它结合于活性中心以外的部位。在测定环胍苯砒抑制 AChE 的 pI_{50} 时,发现它和底物同时加酶解温所测得的 pI_{50} 与它预先和酶解温 15 min,然后加底物解温所测得 pI_{50} 值相同,说明底物和环胍苯砒没有竞争作用。也就是说,环胍苯砒不象 ACh 那样作用于 AChE 的活性中心,而可能是结合于 AChE 活性中心以外的部位,或叫外周阴离子部位或叫变构部位⁽⁷⁾。环胍苯砒也可能是一个 AChE 的变构剂,但有待更多的研究。

致谢 环胍苯砒由本所董永明组合成。本研究得到孙曼霁和方允中两位研究员的有益指点。

REFERENCES

- 1 Capps DB, Bird OD, Elsjager EF, et al. 1-Aryl-4, 6-diamino-1,2-dihydro-s-triazines. Contrasting effects on intestinal helminths, bacteria, and dihydrofolic reductase (1,2). *J Heterocyclic Chem* 1968; 5 : 355

- 2 Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; 7 : 88
- 3 de la Huerga J, Yesinick C, Popper H. Colorimetric method for the determination of serum cholinesterase. *Am J Clin Pathol* 1952; 22 : 1126
- 4 Augustinsson K-B. Assay methods for cholinesterases. Edited by David Glick. *Methods of biochemical analysis*: vol 5. NY: Interscience, 1957 : 14-56
- 5 Dixon M. The determination of enzyme inhibitor constants. *Biochem J* 1953; 55 : 170
- 6 Silver A. *The biology of cholinesterases*. 1st ed. Amsterdam: North-Holland, 1974 : 32-7
- 7 Roufogalis BD, Quist EE. Relative binding sites of pharmacologically active ligands on bovin erythrocyte acetylcholinesterase. *Mol Pharmacol* 1972; 8 : 41
- 8 Stein HH, Lewis GJ. Noncompetitive inhibition of acetylcholinesterase by eserine. *Biochem Pharmacol* 1969; 18 : 1697
- 9 Wang YE, Yue DX, Tang XC. Anti-cholinesterase activity of huperzine A. *Acta Pharmacol Sin* 1986; 7 : 110
- 10 Hao XY, Qin BY. Effects of soman, VX and 5-(1,3,3-trimethyl) indolonyl-N, N-dimethyl carbamate on cholinesterase isoenzymes in mouse plasma. *Ibid* 1986; 7 : 303
- 11 Liu W, Tsou CL. Determination of rate constants for the irreversible inhibition of acetylcholine esterase by continuously monitoring the substrate reaction in the presence of the inhibitor. *Biochim Biophys Acta* 1986; 870 : 185
- 12 Forsberg A, Gertrud PUU. Kinetics for the inhibition of acetylcholine esterase from the electric eel by some organophosphates and carbamates. *Eur J Biochem* 1984; 140 : 153

中国药理学报 *Acta Pharmacologica Sinica* 1990 Mar; 11 (2) : 130-133

麻黄碱对离体豚鼠门静脉突触后 α -受体和突触前 β -受体的作用¹

包建新²、王斌³、杨藻宸 (上海医科大学基础医学部药理研究室, 上海 200032, 中国)

Effects of ephedrine on postsynaptic α -adrenoceptors and presynaptic β -adrenoceptors in isolated guinea pig portal veins

BAO Jian-Xin, WANG Bin, YANG Zao-Chen
(Department of Pharmacology, Faculty of Basic Medical Sciences, Shanghai Medical University, Shanghai 200032, China)

ABSTRACT. The effects of ephedrine (Eph) were compared with those of tyramine (Tyr)

and phenylephrine (Phe) in ring segments of guinea pig portal vein *in vitro*. Eph (3-1000 $\mu\text{mol/L}$), Tyr (10-1000 $\mu\text{mol/L}$) and Phe (1-1000 $\mu\text{mol/L}$) all produced concentration-dependent contractile responses, which were exceedingly depressed by α -adrenoceptor blocker phentolamine (31 $\mu\text{mol/L}$). Pretreatment with reserpine 1 mg/(kg·d) \times 2 d markedly diminished the effect of Tyr, but greatly potentiated the effects of Eph and Phe. Both Eph (1-30 $\mu\text{mol/L}$) and Tyr (10-100 $\mu\text{mol/L}$), but not Phe, significantly increased the electrical field stimulation (duration 2 ms, 3 Hz, 10 s, 50 V, 10 min intervals) evoked contractions of the portal veins. β -Adrenoceptor blocker propranolol (0.5 $\mu\text{mol/L}$) greatly inhibited this effect of Eph, without affecting that of Tyr. It is suggested that the effect of Tyr is mainly due to its release of endogenous norepinephrine (NE) from the nerve terminals; conversely, Eph mainly acts on postsynaptic α -adrenoceptors directly with some NE-releasing action which may involve the activation of presynaptic β -adrenoceptors.

KEY WORDS ephedrine; portal vein; alpha

Received 1989 Feb 2 Accepted 1989 Sep 1

¹ Project supported by the Science Fund of the Chinese Academy of Sciences, No Bio-365 and presented at the 5th Southeast Asian and Western Pacific Regional Meeting of Pharmacologists, Beijing, 1988 Jul 4-8.

² Now in Department of Physiology I, Karolinska Institutet, S-104 01, Stockholm, Sweden

³ Now in Department of Forensic Medicine, Wannan Medical College, Wuhu 241000, China