

0.4 %  $\pm$  s 0.5 % 和 26 %  $\pm$  s 13 % , 但两者无性别差异。结论: 右美沙芬代谢的表型分型为中国

人异喹肼 4-位羟化 (CYP2D6) 多态性提供了新的信息。

BIBLID: ISSN 0253-9756

Acta Pharmacologica Sinica 中国药理学报

1997 Sep; 18 (5); 444 - 446

## Anionic subsite of active center of *Torpedo* acetylcholinesterase constructs a part of its conformational epitope<sup>1</sup>

FU Feng-Hua, XIN Yan-Bin<sup>2</sup>, LI Feng-Zhen<sup>3</sup>, ZHANG Han<sup>3</sup>, SUN Man-Ji<sup>3,4</sup>

(Department of Immunology, Binzhou Medical College, Binzhou 256603;

<sup>2</sup>Institute of Microbiology and Epidemiology,

<sup>3</sup>Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences, Beijing 100850, China)

**KEY WORDS** *Torpedo*; acetylcholinesterase; monoclonal antibodies; epitope; structure-activity relationship; pralidoxime compounds

**AIM:** To study the structure-activity relationship of *Torpedo* acetylcholinesterase (AChE) and explore whether the anionic subsite of the active center is a constituent of the conformational epitope of enzyme.

**METHODS:** Using ELISA and enzyme inhibition test to examine the effect of 1-methyl-2-hydroxyiminomethylpyridium chloride (2-PAM), an anionic subsite probe of AChE, on the immunoreactivity between *Torpedo* AChE and its monoclonal antibody (McAb) 3F3. **RESULTS:** McAb 3F3 did not react with 2-PAM-AChE complex. 2-PAM decreased the inhibitory rate of McAb 3F3 on AChE in a concentration-dependent fashion, but did not dissociate the McAb 3F3-AChE complex. **CONCLUSION:** Anionic subsite of the active center of *Torpedo* AChE constructs a part of its conformational epitope.

Acetylcholinesterase (EC 3. 1. 1. 7, AChE) is normally associated with the breakdown of acetylcholine in the synapse and the neuro-muscular junction. Among monoclonal antibodies (McAb) against *Torpedo* AChE<sup>[1]</sup>, only McAb 3F3 was able to

combine with the conformational epitope of AChE active center<sup>[2]</sup>. 2-PAM is a good probe for the anionic subsite of AChE owing to its hydrophobic pyrimidine ring and N-CH<sub>3</sub> substituent causing molecular orbit interaction via  $\pi - \pi$  charge translocation with aromatic residues lining the surface of the active site gorge. In this paper the effect of 2-PAM on the binding of McAb 3F3 with AChE was studied to find whether the anionic subsite of the active center of *Torpedo* AChE constructs a part of its conformational epitope.

### MATERIALS

*Torpedo* AChE was purified from electric organ of *Torpediniforms Torpedo torpedo* by affinity chromatography<sup>[3]</sup>. McAb 3F3 in the ascites fluids produced in Balb/c mice injected with its corresponding hybridoma cells was purified by protein-A affinity-chromatography<sup>[1,4]</sup>. Horseradish peroxidase-labeled goat-anti-mouse IgG was purchased from Beijing Biotinge Biomedicine Co.

Acetylcholine bromide (ACh·Br) was purchased from Beijing Chemical Plant. 2-PAM was synthesized in Institute of Pharmacology and Toxicology (purity 98 %). Other chemicals were all of AR.

### METHODS AND RESULTS

**Influence of 2-PAM on the immunoreaction between McAb 3F3 and AChE** Aliquots (100  $\mu$ L/well) of *Torpedo* AChE (5 mg  $\cdot$  L<sup>-1</sup>) in *Torpedo* saline (phosphatidylcholine 0.01 %, NaCl 1

<sup>1</sup> Project supported by the National Natural Science Foundation of China, No 38970211.

<sup>4</sup> Correspondence to Prof SUN Man-Ji. P.hn: 86-10-6689-8336.

Fax: 86-10-6821-1656. E-mail: sunmj@nic.bmi.ac.cn

Received 1996-11-14

Accepted 1997-04-30

mol·L<sup>-1</sup>, KCl 5 mmol·L<sup>-1</sup>, CaCl<sub>2</sub> 4 mmol·L<sup>-1</sup>, MgCl<sub>2</sub> 2 mmol·L<sup>-1</sup>, pH 8.0) containing 2-PAM 10 mmol·L<sup>-1</sup> were coated onto microplate at 4 °C for 24 h. McAb 3F3 was used as the first antibody and horseradish peroxidase-labelled goat-anti-mouse IgG as the second antibody. ELISA was performed in the conventional way<sup>[5]</sup>. The results showed that McAb 3F3 was not able to immunoreact with AChE molecule bearing with 2-PAM (Tab 1).

**Tab 1. Immunoreaction between McAb 3F3 and 2-PAM-AChE complex.** *n* = 3 experiments of ELISA.  $\bar{x} \pm s$ . \**P* < 0.01 vs native AChE. Negative control: mouse non-specific IgG 5 g·L<sup>-1</sup>.

Antigen	Absorbance at 492 nm	
	3F3	Negative control
Native AChE	0.36 ± 0.09	0.006 ± 0.006
2-PAM-AChE	0.014 ± 0.016 <sup>c</sup>	0.006 ± 0.006

**Influence of 2-PAM on the enzyme inhibition of AChE by McAb 3F3** Enzyme activity of diluted AChE (1 : 50, vol/vol, with *Torpedo*-saline) was assayed colorimetrically<sup>[6]</sup>. The AChE activity was proportional to the amount of enzyme used below 6 μL. McAb 3F3 (20 μL) and AChE (4 μL) were mixed and made up to a total volume of 50 μL with *Torpedo*-saline at 37 °C for 1 h. AChE activity was determined and the inhibition rate was calculated. It showed that 70 % of AChE activity was inhibited by McAb 3F3 20 μL (Tab 2).

**Tab 2. Influence of 2-PAM on the inhibition of AChE by 3F3.** *n* = 3 experiments of ELISA,  $\bar{x} \pm s$ . \**P* > 0.05, <sup>b</sup>*P* < 0.05, <sup>c</sup>*P* < 0.01 vs no 2-PAM. Normal control of AChE activity without McAb 3F3 and 2-PAM: 277 ± 4.

2-PAM/ mmol·L <sup>-1</sup>	Activity of AChE/ nmol ACh in 30 min	Inhibition of AChE/ %
0	84 ± 20	70
0.1	96 ± 7 <sup>a</sup>	66
0.5	133 ± 9 <sup>b</sup>	52
1.0	161 ± 13 <sup>c</sup>	42
5.0	180 ± 5 <sup>c</sup>	35

To observe the influence of 2-PAM on the inhibition of AChE by McAb 3F3, AChE (4 μL) was first incubated with 2-PAM (5 μL) in *Torpedo*-saline

(21 μL) at 37 °C for 10 min, then McAb 3F3 (20 μL) was added and incubated continuously for 1 h. Finally, the catalytic activity of AChE was determined as above. The results showed that along with the increase of the concentration of 2-PAM the inhibitory rate of McAb 3F3 to AChE decreased significantly, 2-PAM impeded the combination between the antigen (AChE) and monoclonal antibody (3F3)(Tab 2).

**Influence of 2-PAM on the 3F3-AChE complex** AChE (4 μL), 3F3 (20 μL), and *Torpedo* saline (21 μL) were mixed at 37 °C for 1 h to form a 3F3-AChE antibody-antigen complex. Then, 2-PAM 5 μL was added to a final concentration of 1 mmol·L<sup>-1</sup>. After incubation at 25 °C, the AChE activity was assayed at different intervals for calculating the inhibitory rate. The results showed that in the presence of 2-PAM 1 mmol·L<sup>-1</sup> the activity of AChE inhibited by 3F3 was not markedly changed (Tab 3).

**Tab 3. Influence of 2-PAM on 3F3-AChE antibody-antigen complex.** *n* = 3 experiments of ELISA.  $\bar{x} \pm s$ . \**P* > 0.05 vs no 2-PAM. Normal control of AChE activity without McAb 3F3 and 2-PAM: 214 ± 25. The AChE activity in presence of 2-PAM 1 mmol·L<sup>-1</sup>: 207 ± 8. The inhibition rate of 2-PAM on AChE was 3 %.

Reaction time/ min	AChE activity/ nmol ACh in 30 min	Inhibition/ %
0	67 ± 6	69
20	71 ± 9 <sup>a</sup>	67
40	74 ± 7 <sup>a</sup>	65
60	65 ± 5 <sup>a</sup>	70
90	74 ± 4 <sup>a</sup>	65
120	80 ± 20 <sup>a</sup>	63

## DISCUSSION

2-PAM is known to bind with the anionic subsite of the active center and its combination with AChE impedes the immunoreaction between AChE and its McAb 3F3, implying that the epitope directed by McAb 3F3 covers the anionic subsite of *Torpedo* AChE active center. The result that 2-PAM decreases the inhibitory rate of McAb 3F3 to AChE further indicates that the McAb 3F3-directed epitope covers the anionic subsite. It also supports that McAb 3F3 competes the 2-PAM binding site at the active center. The data mentioned show that the anionic subsite of the active center of *Torpedo* AChE constructs a part of the

conformational epitope of the enzyme molecule.

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电鳐乙酰胆碱酯酶活性中心负矩部位构成其构象决定簇的一部分<sup>1</sup>

R992

傅风华, 辛颜彬<sup>2</sup>, 李凤珍<sup>3</sup>, 张翰<sup>3</sup>, 孙曼霖<sup>3,4</sup>  
(滨州医学院免疫学教研室, 滨州 256603; 军事医学科学院<sup>2</sup>微生物与流行病学研究所, <sup>3</sup>毒物药物研究所, 北京 100850; 中国)

关键词 电鳐属; 乙酰胆碱酯酶; 单克隆抗体; 抗原决定簇; 结构-活性关系; 解磷定化合物

目的: 研究电鳐乙酰胆碱酯酶(AChE)结构功能关系, 探讨 AChE 负矩部位是否是其构象决定簇的一个组成部分. 方法: 用 ELISA 及酶抑制实验观察 AChE 负矩部位探针 2-PAM 对 AChE 与其 McAb 3F3 之间的免疫反应性的影响. 结果: McAb 3F3 不能与 2-PAM 及 AChE 的复合物反应; 2-PAM 浓度依赖性地降低 McAb 3F3 对 AChE 的抑制率; 但不能解离 AChE 与 3F3 构成的抗原抗体复合物. 结论: 电鳐 AChE 活性中心负矩部位构成其活性中心构象抗原决定簇的一部分.

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