BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jul; 17 (4): 369 - 372

Inhibitory effect of suberogorgin on acetylcholinesterase

PENG Wen-Duo, XU Shi-Bo, PENG Xun[1]
(*Pharmacology Laboratory, Department of Biology, Sun Yat-Sen University, Guangzhou 510275, China*)

KEY WORDS suberogorgin; acetylcholinesterase; butyrylcholinesterase; erythrocyte membrane; brain; muscles

AIM: To study the selection, reversibility, and kinetics of suberogorgin (Sub) on acetylcholinesterase (AChE). **METHODS**: The human plasma was used as butyrylcholinesterase (BuChE). The activity of ChE was determined with colorimetry. **RESULTS**: Sub obviously inhibited the AChE *in vitro* with pI_{50} 4.03, 4.92, 3.82, and 4.67 in RBC membranes (of rat and human) and tissue extracts (of rat brain and earthworm dorsal muscle), respectively. No inhibition on BuChE was observed. The inhibition of Sub on AChE was far lower than that of physostigmine, but was close to that of galanthamine. Sub decreased the AChE activity to the lowest within 3 min after it was incubated with AChE. Centrifugalization washing reactivated the AChE which had been inhibited by Sub. The double-reciprocal plots of different concentrations of Sub on AChE showed parallel lines. **CONCLUSION**: Sub was a selective, reversible, and contra-competetive inhibitor of AChE at the binding site on the peripheral anion region of AChE.

Suberogorgin (Sub) was isolated from *Gorgoniae suberogorgia* sp from South China Sea. Its pharmacological actions were not related to α, β, H_1 receptors or Ca^{2+}, Na^+ channels, but inhibited acetylcholinesterase (AChE)[1]. To understand the inhibitory characteristics of Sub, we investigated its selection, reversibility, and kinetics on AChE from various tissues.

MATERIALS AND METHODS

Sub, spectrum pure, was isolated by Department of Chemistry, Sun Yat-Sen University; sodium cholate and physostigmine salicylate (Phys) (Serva, USA); galanthamine hydrobromide (Gal) (Suzhou Sixth Pharmaceutical Factory, China); acetylthiocholine iodide (ATCh) and butyrylthiocholine iodide (BuTCh) (Fluka, Switzerland); 5, 5′-dithio-2, 2′-dinitrobenzoic acid (DTNB) (Shanghai Institute of Biochemistry, Chinese Academy of Sciences); sodium dodecyl sulphate (SDS) (BDH, Great Britain).

Preparation of tissue sample of AChE

1 **RBC membrane** Sprague-Dawley rats (n = 10) weighing 268 ± s 15 g of either sex were bred in our laboratory. Rat blood was collected from aorta after anesthesia. Human blood was obtained from The Second Affiliated Hospital of Guangzhou Medical College. Blood was centrifugalized at 2500 × g, and the precipitate was centrifugalized and washed with phosphate 5 mmol·L^{-1} buffer (pH 7.4) containing NaCl 0.15 mol·L^{-1} for 3 times at 4 ℃. The RBC membrane was prepared by the method [2].

2 **Extract of rat brain** Brain besides cerebellum was homogenized with cold sucrose 0.32 mol·L^{-1} solution. The homogenate was centrifugalized at 2700 × g at 4 ℃. The supernatant was blended with phosphate 25 mmol·L^{-1} buffer (pH 7.4) at 1:1 (vol/vol) and sodium cholate 116 mmol·L^{-1} solution at 9:1 (vol/vol) at 0 - 2 ℃. Six hours later, the mixture was centrifugalized at 20 000 × g for 45 min at 4 ℃, and the supernatant was kept at − 20 ℃.

3 **Extract of earthworm dorsal muscle** Earthworm (*Eisenia foetida*) dorsal muscles, prepared by the method[3], were homogenized with sucrose 0.32 mol·L^{-1} solution at 1:4 (wt/vol) in ice-water. The homogenate was centrifugalized at 14 000 · g for 20 min at 4 ℃, and then the supernatant was kept at − 20 ℃.

Preparation of BuChE Human plasma was used as BuChE[2].

Determination of the activity of ChE The activity of ChE was determined by colorimetry[1,4]. The volume of reacted liquid of ChE was 4 mL containing ATCh 0.3 or BuTCh 0.4 mmol·L^{-1}, ChE 0.1 or 0.2 mL, and test drug. After the reacted liquid was incubated at 37.5 ℃ for 3 min, 1 mL of SDS 0.12 mol·L^{-1} and 1 mL of DTNB 5 mmol·L^{-1} were added. The absorbance of reacted liquid was recorded on 721A spectrometer at 440 nm.

RESULTS

Inhibition of Sub on ChE *in vitro* Sub obviously inhibited the activities of AChE from

[1] Now in *Cancer Hospital, School of Medicine, Shantou University, Shantou 515031, China*.

Received 1994-03-22 Accepted 1995-06-23

various tissues (Fig 1). Its pI_{50} (negative logarithm of molar concentration causing 50 % inhibition of ChE) was 3.82 - 4.92 (Tab 1). The inhibition of Sub on AChE was far weaker than that of Phys, but was close to that of Gal. No inhibition of Sub on BuChE was observed, while the pI_{50} of Phys on BuChE was 7.10.

Tab 1. pI_{50} of suberogorgin (Sub), galanthamine (Gal), and physostigmine (Phys) on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) from tissues. $n = 10$.

	Sub	Gal	Phys
AChE			
Rat RBC membrane	4.03 (3.98 - 4.09)	4.54 (4.48 - 4.60)	6.94 (6.87 - 7.00)
Human RBC membrane	4.92 (4.85 - 4.98)		7.55 (7.46 - 7.65)
Rat brain	3.82 (3.75 - 3.88)	4.38 (4.31 - 4.46)	6.68 (6.57 - 6.78)
Earthworm muscle	4.67 (4.60 - 4.73)		6.47 (6.41 - 6.53)
BuChE			
Human plasma			7.10 (7.00 - 7.20)

Inhibitory characteristics of Sub on AChE

1 Reversibility After the RBC membrane or the extract of rat brain was incubated with Sub or Phys at 37.5 ℃, all the activities of AChE were decreased to the lowest within 3 min (Fig 2). Moreover, in rat RBC membrane, centrifugalization washing made the activities of AChE, which had been inhibited by Sub 0, 10, 80, and 160 μmol·L^{-1}, return to 100 ± 11 %, 99 ± 2 % ($P > 0.05$), 97 ± 1 % ($P > 0.05$), and 94 ± 5 % ($P > 0.05$), respectively; but made the activities of AChE which had been inhibited by Phys 0.08 and 2.56 μmol·L^{-1} only return to 64 ± 7 % and 49 ± 10 % (all $P < 0.01$) respectively.

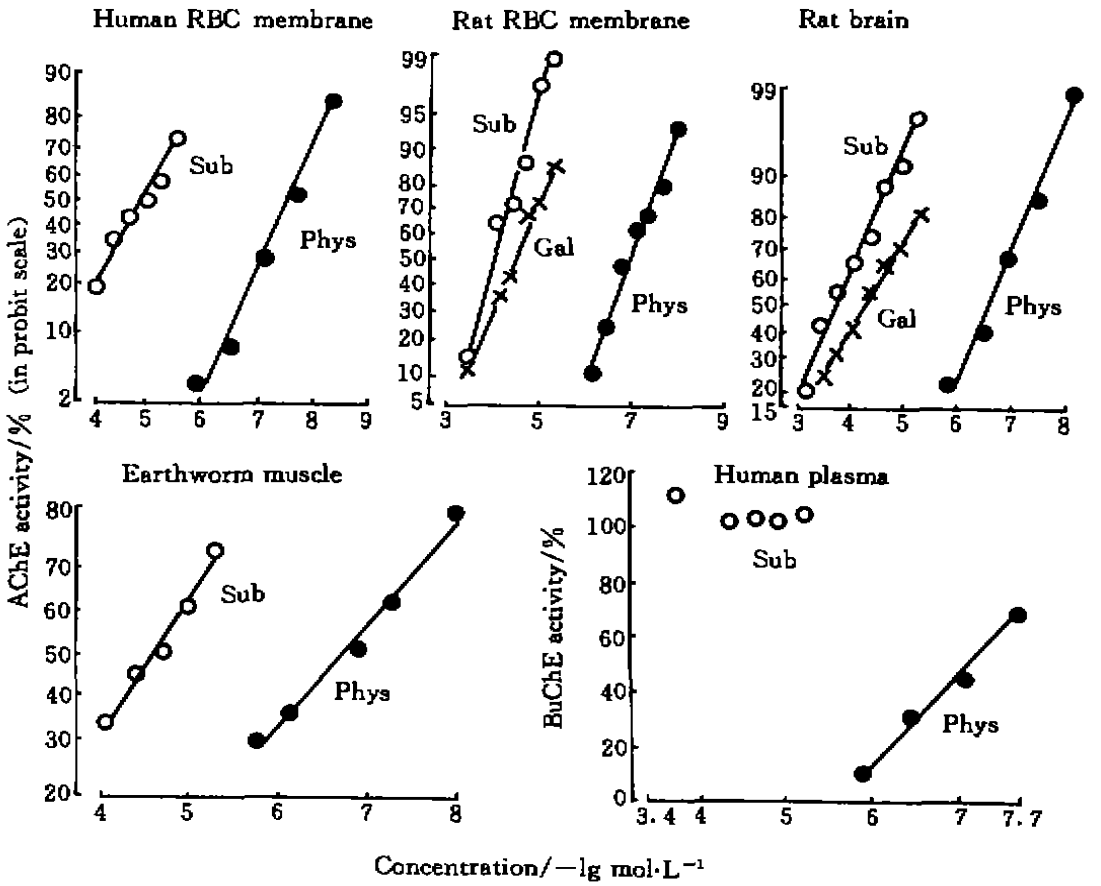

Fig 1. Inhibitions of suberogorgin (Sub), galanthamine (Gal), and physostigmine (Phys) on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). $n = 10$.

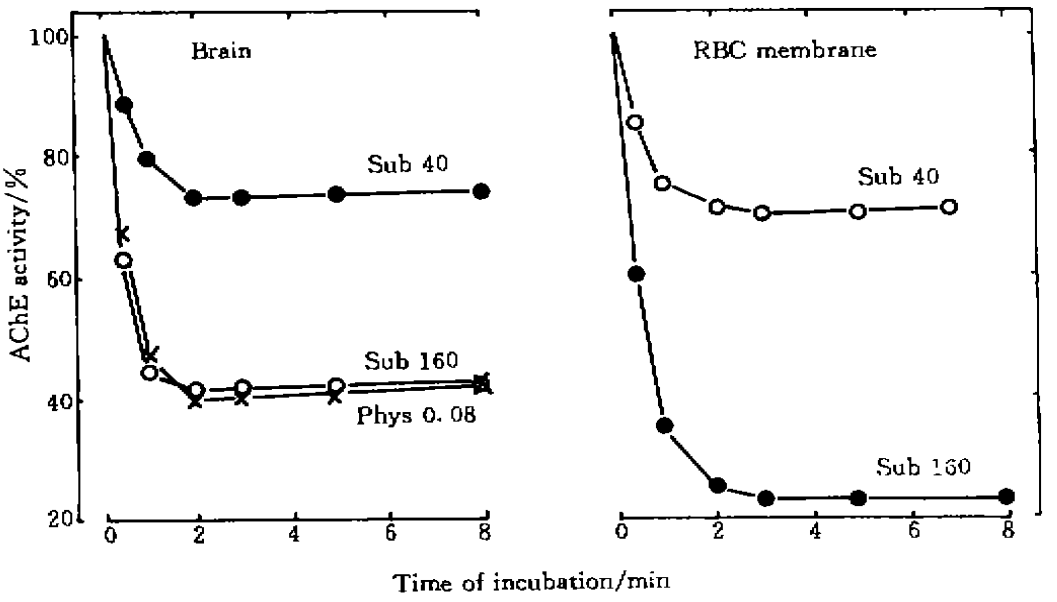

Fig 2. AChE activities after incubation with different concentrations ($\mu mol \cdot L^{-1}$) of Sub or Phys. $n = 7$.

2 Kinetics In human RBC membrane or extract of rat brain, the Lineweaver-Burk double-reciprocal plots of Sub showed parallel lines (contra-competitive plots) (Fig 3).

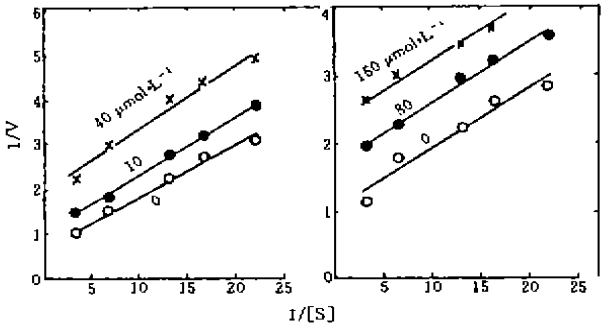

Fig 3. Double-reciprocal plots of AChE. $n = 9$.

In addition, the pI_{50} of Sub, which was determined by the method that Sub had been incubated with human RBC membrane for 10 min and then incubated with ATCh, was 4.93 (4.86 – 5.00). It is identical to above result ($pI_{50} = 4.92$). This showed that there was no competitiveness between Sub and substrate.

DISCUSSION

Our experiments showed that Sub was a selective AChE-inhibitor. This selection was far higher than that of Phys, and also higher than that of huperzine A [5] and 5-(1,3,3-trimethyl)indolinyl-*N*,*N*-dimethyl carbamate[6].

The facts that Sub contra-competitively inhibited the AChE, and there was no competitiveness between Sub and substrate, showed that the binding site between Sub and AChE was on the peripheral region of AChE (peripheral anion region [7]). Because the peripheral anion region do not exist in BuChE[8], Sub did not inhibit the BuChE.

Actually, the structure of peripheral anion region is a carboxyl[8] which can bind with the carboxyl of Sub in a hydrogen bond in physiological solution (Fig 4).

This kind of binding is not as firm as the covalent-bond-binding between Phys and the active center of AChE. Therefore, the activity of AChE, inhibited by Sub, was easier to recover than that by Phys, after centrifugalization washing. These results, together with the observations that Sub decreased the activities of AChE to the lowest within 3 min after it was incubated with the tissue samples, unlike the actions of soman[7] on AChE, indicated that the inhibition of Sub on AChE might be reversible.

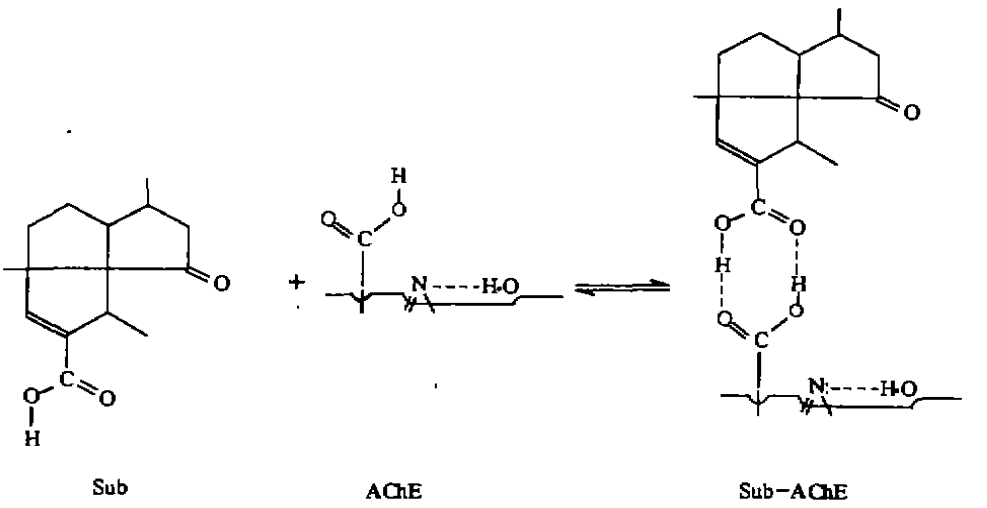

Fig 4. A possible binding between Sub and AChE in physiological solution.

REFERENCES

1 Xu SB, Peng WD, Hu YT, Wang YF. Excitant effect of sodium suberogorgin on isolated rabbit ileum. Acta Pharmacol Sin 1992; 13: 459 - 63.

2 Xu H, Tang XC. Cholinesterase inhibition by huperzine B Acta Pharmacol Sin 1987; 8: 18 - 22.

3 Zhou TC, Zhou JQ. Earthworm dorsal muscle preparation for studying gammaaminobutyric acid, acetylcholine and glutamic acid. Acta Pharmacol Sin 1981; 2: 145 - 8.

4 Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88 - 95.

5 Wang YE, Yue DX, Tang XC. Anti-cholinesterase activity of huperzine A. Acta Pharmacol Sin 1986; 7: 110 - 3.

6 Hao XY, Qin BY. Effects of soman, VX and 5-(1, 3, 3-trimethyl) indolinyl-*N*, *N*-dimethyl carbamate on cholinesterase isoenzymes in mouse plasma. Acta Pharmacol Sin 1986; 7: 303 - 7.

7 Yuan BJ, Qin BY. Inhibitory properties of cycloguanide phenylsulfone on acetylcholinesterase. Acta Pharmacol Sin 1990; 11: 126 - 30.

8 Silver A. The biology of cholinesterase. 1st ed. Amsterdam: North-Holland 1974: 332 - 7.

36P-372

柳珊瑚酸对乙酰胆碱酯酶的抑制作用

彭汉铎，许实波，彭 迎[1]
(中山大学生物系药理室，广州 510275，中国)

关键词 柳珊瑚酸；乙酰胆碱酯酶；丁酰胆碱酯酶；红细胞膜；脑；肌肉

A **目的**：研究柳珊瑚酸(suberogorgin, Sub)抗乙酰胆碱酯酶(AChE)作用的选择性、可逆性和动力学. **方法**：制备大鼠和人红细胞膜、大鼠脑和蚯蚓背肌提取液作为 AChE 组织样品，人血浆为丁酰胆碱酯酶(BuChE)样品；比色法测酶活力. **结果**：Sub 明显抑制上述样品的 AChE 活力，其 pI_{50} 各为 4.03，4.92，3.82 和 4.67，对 BuChE 无抑制作用. Sub 与 AChE 一起孵温后，酶活力在 3 min 内降至最低，而且离心洗涤可使酶活力恢复. 不同浓度的 Sub 对 AChE 抑制作用的动力学曲线为平行线. **结论**：Sub 是选择性和可逆性的 AChE 抑制剂，它与 AChE 的结合点在 AChE 的外周阴离子部位.

R965.1
R964