去甲肾上腺素；节后交感神经纤维；组胺 H_3 受体；高压液相色谱法

目的：观察组胺 H_3 受体介导抑制心交感神经末稍释放去甲肾上腺素(NE)。方法：采用 HPLC-ECD 法检测电场刺激(50 mA，5 ms)诱发交感神经末稍释放 NE。结果：α-甲基组胺浓度依赖性抑制电场刺激诱发的 NE 释放。H_1，H_2，α_2，β_2 受体拮抗剂不能拮抗 α-甲基组胺的作用。Thioperamide 浓度依赖性拮抗 α-甲基组胺的作用　单独应用 thioperamide 可促进 NE 释放　结论：突触前组胺 H_3 受体参与介导抑制心交感神经末稍释放 NE。

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Effects of HI-6 on muscle acetylcholine receptor: analysis on minimal reaction model

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KEY WORDS HI-6; skeletal muscle; cholinergic receptors; messenger RNA; *Xenopus laevis*; oocytes

AIM: To study the action of 1-(2-hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI-6) on skeletal muscle acetylcholine receptor (N_2-ChR). **METHODS**: N_2-ChR was expressed in *Xenopus laevis* oocyte after injection with mRNA extracted from denervated rat leg muscles. The inward membrane currents induced by various concentrations of carbamylcholine and effects of HI-6 or *d*-tubocurarine on the currents were measured with voltage clamp technique by fast cell flow of agents. The actions of HI-6 and *d*-tubocurarine on N_2-ChR were analyzed by using the minimal reaction model. **RESULTS**: K of 40.05, 156.00, and 334.67 μmol·L^{-1} for HI-6, K of 0.02, 0.10, and 0.18 μmol·L^{-1} for *d*-tubocurarine were obtained by using the competing for single acetylcholine (ACh)-binding site model, the competing for two ACh-binding sites model, and the noncompetitive inhibition model, respectively. **CONCLUSION**: HI-6 and *d*-tubocurarine competed for two ACh-binding sites of N_2-ChR with equal affinity to antagonize the effects of the agonist on N_2-ChR. The N_2-ChR inhibition by HI-6 is much weaker than that by *d*-tubocurarine.

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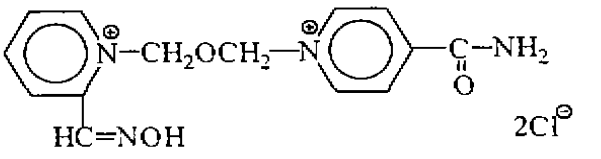
Oximes acetylcholinesterase reactivators such as 1-(2-hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI-6) and 1,1′-[Oxybis(methylene)]bis-[(4-hydroxyimino)methyl]-pyridinium dichloride (obidoxime chloride) together with certain cholinergic blockers have been used effectively to antagonize organophosphorus agent (OP)-poisoning. However, the acetylcholinesterase reactivation mechanism alone is not sufficient to explain the antidotal effect of oximes against OP-induced toxicity[1]. Some other mechanisms such as antimuscarinic, ganglion-blocking, neuromuscular-blocking properties, and acetylcholinesterase-like activity may also contribute to their antidotal effect. The effects of oximes reactivators on N_2-ChR is quite complicated, including curarine-like inhibition[2], noncompetitive antagonism of N_2-ChR and excitatory actions of N_2-ChR[3]



1-(2-Hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI-6)

In the present study, the effects of HI-6 and *d*-tubocurarine on N_2-ChR expressed in *Xenopus laevis* oocytes were investigated to reveal the mechanism of their action on N_2-ChR.

MATERIALS AND METHODS

Expression of N_2-ChR N_2-ChR mRNA was extracted from denervated *extensor digitorum longus* and *soleus* muscles of adult Wistar rat (Laboratory Center of this University)[4]. The oocytes were prepared from ♀ *Xenopus laevis* (Institute of Developmental Biology, Chinese Academy of Sciences) and injected with 20 – 50 nL of mRNA (0.8 – 1.0 $g \cdot L^{-1}$)[5] and incubated in Bath's solution containing streptomycin 50 $mg \cdot L^{-1}$, penicillin 100 $kU \cdot L^{-1}$, and 0.6 % horse serum at 13 – 18 ℃ for 3 – 10 d.

Measurement of membrane current The inward membrane currents evoked by carbamylcholine were measured according to Ref 6 with slight modification: the agents were administered through a U-tube with one hole (200 μm in diameter).

To study the effects of HI-6 (Academy of Military Medical Science, China) and *d*-tubocurarine (Sigma) on carbamylcholine and minimize desensitization of N_2-ChR caused by them, both were applied with carbamylcholine (Sigma) simultaneously.

Data analysis The responses induced by various concentrations of carbamylcholine were usually normalized to those at 5 $mmol \cdot L^{-1}$. The results were analysed according to the minimal reaction model[7-9]: in the absence of antagonist, the relationship between the peak current amplitude ($\tilde{I}$ and I) evoked by agonist and L (ligand concentration) was given by Eq 1 and Eq 2. The value of 143 nA for $I_M R_M$ was obtained by assuming the value of P_O as 0.7 at 5 $mmol \cdot L^{-1}$ of carbamylcholine.

$$\tilde{I} = \frac{I_M R_M L^2}{L^2(1+\Phi) + 2K\Phi L + K^2\Phi} \quad (1)$$

$$I = \frac{I_M R_M L^2}{L^2(1+\Phi) + 2K\Phi L + K^2\Phi} \cdot \frac{K_R}{K_R + L} \quad (2)$$

$$\tilde{I}/I = 1 + L/K_R \quad (3)$$

Competitive inhibition model: (1) Competing for single ACh-binding site model: In the presence of competitive antagonist (B) assumed to bind one of the two ACh-binding sites with equal affinity, characterized by dissociation constant K_C, the relationship between I and L was given by Eq 4.

$$I_C = \frac{I_M R_M L^2}{L^2(1+\Phi) + 2K\Phi L + K^2\Phi + (K\Phi L + K^2\Phi)\cdot B/K_C} \cdot \frac{K_R}{K_R + L} \quad (4)$$

$$\frac{I}{I_C} = 1 + \frac{B}{K_C} \cdot \frac{K\Phi L + K^2\Phi}{L^2(1+\Phi) + 2K\Phi L + K^2\Phi} \quad (5)$$

(2) Competing for two ACh-binding sites model: In the presence of the antagonist assumed to compete for two ACh-binding sites with equal affinity, characterized by the dissociation constant K_{2C}, the relationship between I and L was given by Eq 6.

$$I_{2C} = \frac{I_M R_M L^2}{L^2(1+\Phi) + 2K\Phi L(1 + B/K_{2C}) + K^2\Phi(1 + B/K_{2C})^2} \cdot \frac{K_R}{K_R + L} \quad (6)$$

$$\frac{I}{I_{2C}} = 1 + \frac{2K\Phi L + 2K^2\Phi}{L^2(1+\Phi) + 2K\Phi L + K^2\Phi} \cdot \frac{B}{K_{2C}} + \frac{K^2\Phi}{L^2(1+\Phi) + 2K\Phi L + K^2\Phi} \cdot \left(\frac{B}{K_{2C}}\right)^2 \quad (7)$$

Noncompetitive inhibition model: The antagonist was assumed to bind a noncompetitive site distinct from the regulatory ACh-binding site, which was characterized by dissociation constant K_{NC}. Eq 8 was the expression for I for this mechanism.

$$I_{NC} = \frac{I_M R_M L^2}{(L^2(1+\Phi) + 2K\Phi L + K^2\Phi)(1 + B/K_{NC})} \cdot \frac{K_R}{K_R + L} \quad (8)$$

$$I/I_{NC} = 1 + B/K_{NC} \quad (9)$$

RESULTS

Dose-dependence of response to carbamylcholine At low carbamylcholine concentrations ($L \ll K_R \leqslant 1$ $mmol \cdot L^{-1}$), the regulatory inhibition effect could be neglected. K and Φ value obtained from the linear fit of data in Fig 2 according to Eq 1 was 397.00 $\mu mol \cdot L^{-1}$ and 0.38, respectively. K_R calculated by using Eq 3 and substituting known values of K and Φ was 118.98 $mmol \cdot L^{-1}$. The dose-response curve (Fig 2 a) fitted by using Eq 2

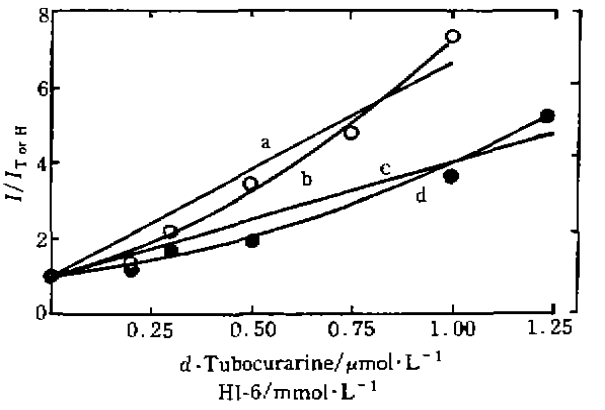


Fig 1. Effects of *d*-tubocurarine (○) and HI-6 (●) on inward membrane current (I) induced by carbamylcholine 1 $mmol \cdot L^{-1}$. a and c were linear fits of data (○) and (●), respectively. b and d were the best fits of data (○) and (●) respectively to Eq 7. $n = 6$ cells.

was similar to that of ACh obtained from *torpedo* N_2-ChR[18].

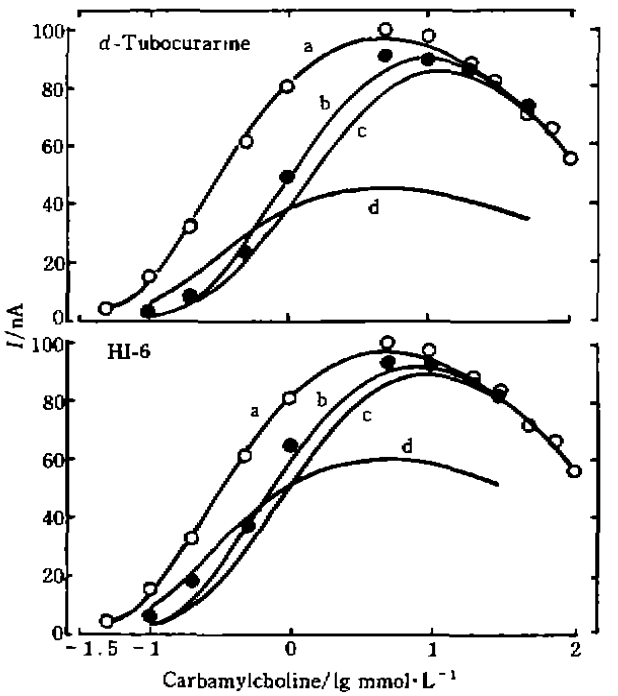


Fig 2. Antagonistic models for *d*-tubocurarine and HI-6 induced by carbamylcholine in the absence (○) and presence (●) of antagonists, analysis on the basic minimal reaction model (a), analysis on competing for 2 ACh-binding sites model (b), for single ACh-binding site model (c), and on the noncompetitive inhibitory model (d). $n = 6-8$ cells.

Mechanism of N_2-ChR inhibition by HI-6

(1) Competing for single ACh-binding site model: K_C for *d*-tubocurarine and HI-6 were 0.02 and 40.05 $\mu mol \cdot L^{-1}$, respectively, which were obtained by using the linear fit equations in Fig 1 (a) ($I/I_T = 1 + 5.606B$) and (c) ($I/I_H = 1 + 2.988B$) and eq 5 in which the expression had been reduced to the eq $I/I_C = 1 + 0.121B/K_C$ by substituting known values of K, Φ, and L (1 $mmol \cdot L^{-1}$). The calculated concentration-response curves for carbamylcholine in the presence of *d*-tubocurarine 0.2 $\mu mol \cdot L^{-1}$ (Fig 2 Ⅰ c) or HI-6 0.2 $mmol \cdot L^{-1}$ (Fig 2 Ⅱ c) obtained by using Eq 4 and substituting values of K, Φ, K_R, and K_C were similar to curve a and the effects were diminished. Both curves were below data sets (Fig 2 Ⅰ & Ⅱ ●) at low carbamylcholine concentrations (< 20 $mmol \cdot L^{-1}$), respectively.

(2) Competing for two ACh-binding sites model: The curves in Fig 1 (b) and (d) represent the best fit of the data to Eq 7 (in which the expression had been reduced to the equation: $I/I_{2C} = 1 + 0.241B/K_{2C} + 0.034\ (B/K_{2C})^2$ by substituting known values of K, Φ, and L (1 $mmol \cdot L^{-1}$), where K_{2C} for *d*-tubocurarine and HI-6 was 0.10 and 156.00 $\mu mol \cdot L^{-1}$, respectively. The calculated concentration-response curves for carbamylcholine in the presence of *d*-tubocurarine 0.2 $\mu mol \cdot L^{-1}$ (Fig 2 Ⅰ b) and HI-6 0.2 $mmol \cdot L^{-1}$ (Fig 2 Ⅱ b) were obtained by using Eq 6 and substituting known values of K, Φ, K_R, and K_{2C}. Two sets of data (Fig 2 Ⅰ & Ⅱ ●) fell on the two curves, respectively.

(3) Noncompetitive inhibition model: The K_{NC} for *d*-tubocurarine and HI-6 obtained by using the linear equations in Fig 1 (a) and (c) and Eq 9 was 0.18 and 334.67 $\mu mol \cdot L^{-1}$, respectively. The calculated concentration-response curves for carbamylcholine in the presence of *d*-tubocurarine 0.2 $\mu mol \cdot L^{-1}$ (Fig 2 Ⅰ d) and HI-6 0.2 $mmol \cdot L^{-1}$ (Fig 2 Ⅱ d) were obtained by using Eq 8 and substituting known values of K, Φ, K_R, and K_{NC}. Neither of the data sets fell on the curves.

DISCUSSION

In this paper, N_2-ChR expressed in oocytes was used to probe the effects of HI-6 and *d*-tubocurarine. The results that the concentration-dependences to carbamylcholine in the presence of HI-6 or *d*-tubocurarine are similar and parallel shift to right from those in the absence of them suggest that both are the competitive antagonists of N_2-ChR. The good fit of the experimental data to the equation for competing for two ACh-binding sites model rather than the other two models indicates that both HI-6 and *d*-tubocurarine bind two competitive ACh-binding sites of N_2-ChR with equal affinity to antagonize the effect of carbamylcholine on N_2-ChR. The much smaller K for *d*-tubocurarine than that for HI-6 indicates that the competitive inhibition of HI-6 on N_2-ChR is much weaker than *d*-tubocurarine.

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以最小反应模型分析 HI-6 抗骨骼肌烟碱受体的作用

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关键词 HI-6；骨骼肌；胆碱能受体；信使 RNA；非洲爪蟾；卵母细胞

目的：研究 1-(2-hydroxyiminomethyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI-6)拮抗骨骼肌烟碱受体(N_2-ChR)的机制。**方法**：将去神经大鼠小腿骨骼肌 N_2-ChR 的 mRNA 注射入非洲爪蟾卵母细胞，表达出功能性 N_2-ChR。以全细胞电压钳和快速全细胞灌流法测定不同浓度氯甲酰胆碱激动 N_2-ChR 产生的内向跨膜电流，以及 HI-6 和筒箭毒碱对其作用的影响。以最小反应模型分析 HI-6 和筒箭毒碱抗 N_2-ChR 的机制。**结果**：分别以竞争双乙酰胆碱结合位点模型，竞争单乙酰胆碱结合位点模型和非竞争性抑制模型分析 HI-6 所得 K 值分别为 156 00，40 05 和 334.67 μmol·L^{-1}，筒箭毒碱的 K 值分别为 0 10，0.02 和 0.18 μmol·L^{-1}。实验所得数据与各模型的配合程度，以竞争双乙酰胆碱结合位点模型最好，而非竞争性抑制模型最差。**结论**：HI-6 与筒箭毒碱属竞争性拮抗作用，尤以竞争双乙酰胆碱结合位点机制更合适，但前者的拮抗作用远较后者弱。

Information for authors

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