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# Facilitatory effect of huperzine-A on mouse neuromuscular transmission *in vitro*

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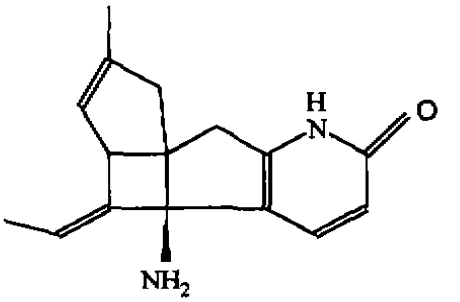
**KEY WORDS** cholinesterase inhibitors; huperzine-A; tacrine; E2020; neuromuscular junction; diaphragm; phrenic nerve; electrophysiology

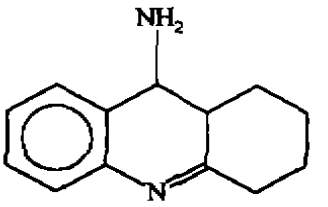
**AIM**: To study the effects of huperzine-A on neuromuscular junction transmission in mouse. **METHODS**: The isolated mouse phrenic nerve-hemidiaphragm preparations were used with the conventional intracellular recording technique. The spontaneous electrical activities of cholinergic nerve terminals (miniature end-plate potentials, MEPP) were recorded. **RESULTS**: Huperzine-A, tacrine, and E2020 at the concentrations of 0.05 – 1 μmol·L$^{-1}$ increased the amplitude, mean rise time, and half decay time of MEPP in a concentration-dependent manner. Their potencies were E2020 > huperzine-A > tacrine. **CONCLUSION**: The anticholinesterase action of huperzine-A in cholinergic synapses is stronger than that of tacrine.

Alzheimer's disease (AD) is concerned with the degeneration of cholinergic neurons in brain[1]. To compensate the cholinergic system, investigation has been carried out with acetylcholinesterase (AChE) inhibitors to increase the acetylcholine (ACh) level in the brain. Such AChE inhibitors do improve symptoms in AD[2]. Tacrine has been approved by FDA in clinical treatment of patients with AD. But its therapeutic usefulness is limited by its hepatotoxicity. E2020 is an effective AChE inhibitor with a long duration of action[3].

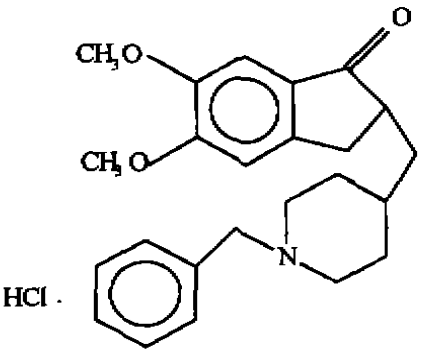
Huperzine-A (Hup-A), a novel alkaloid, is a potent and selective AChE inhibitor[4] showing an increase in ACh level for several hours[5] and fewer side effects than tacrine[6]. It improved clinical manifestations in patients with myasthenia[7]. It made better the learning and memory in mice with higher efficacy than tacrine[8]. In phase II clinical trials Hup-A improved the memory quotient of AD patients with minimal side effects[9]. This study was designed to compare their effects on neuromuscular junction transmission.




**Huperzine-A**




**Tacrine**




**E2020**

## MATERIALS AND METHODS

Hup-A (colorless powder, purity > 98 %) was prepared by Department of Phytochemistry in this Institute. Tacrine was purchased from Sigma Chemical Co. E2020 (colorless powder, purity > 98 %) was kindly provided by Dr WAKAMATSU Takeshi (Tsumura & Co, Japan). Other

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agents were AR.

Kunming strain mice (Clean, Certificate No 005) of either sex weighing 18 - 22 g were decapitated. The left hemidiaphragm with phrenic nerve was mounted in 40 mL standard Krebs solution at 30 - 32 ℃ gassed with 95 % $O_2$ + 5 % $CO_2$[10]. Hup-A, tacrine, or E2020 solution added was prewarmed to 30 - 32 ℃.

Neuromuscular junction was identified by spontaneous miniature end-plate potential (MEPP) using conventional intracellular recording. The microelectrode filled with KCl 3 mol·$L^{-1}$ had a resistance of 5 - 15 MΩ. MEPP, recorded as potential change by a high impedance amplifier (Axoclamp-2B, Axon Instruments Inc), was displayed on a Nicolet 201 oscilloscope (Nicolet Instruments Inc) and stored in parallel on a DTR 1204 recorder (Biologic Instruments). Synaptic potentials were analyzed with an Axotape 2.0 Software (Axon Instruments Inc), and a DigiData 1200 interface (Axon Instruments Inc).

Data in $\bar{x} \pm s$ of at least 8 - 10 end-plates from 3 experiments were compared with ANOVA.

## RESULTS

MEPP in control levels were 0.4 - 0.8 mV in amplitude, 0.13 - 0.20 ms in rise time, and 0.20 - 0.50 ms in half decay time at a testing potential of - 55 mV to - 75 mV. Hup-A, tacrine, or E2020, increased the amplitude, slowed the rise time and half-decay time (Fig 1).

In the concentration range of 0.05 - 1 μmol·$L^{-1}$, Hup-A, tacrine, and E2020 increased MEPP amplitude, rise time and half decay time in a concentration-dependent manner. Hup-A 0.05 μmol·$L^{-1}$ produced a 32 % whereas 1 μmol·$L^{-1}$ produced a 92 % ($P < 0.01$) increase in the amplitude of MEPP. Tacrine 0.05 μmol·$L^{-1}$ hardly changed the rise time of MEPP, while 1 μmol·$L^{-1}$ prolonged MEPP rise time to 162 %. As for E2020, such concentration-dependent relationship was also seen. The anti-AChE action of Hup-A was more potent than that of tacrine, but weaker than that of E2020. At 1 μmol·$L^{-1}$, tacrine increased the amplitude, rise time, and half decay time of MEPP by 170 %, 160 %, and 150%, Hup-A by 190 %, 180 %, and 170 % while E2020 by 210 %, 190 %, and 190 %, respectively.

## DISCUSSION

In the central nervous system at the synaptic level, it is difficult to directly measure ACh release and the mean life of ACh molecules. However, the vertebrate neuromuscular junction where ACh was released in a quantal manner is an alternative experimental model to measure the level of ACh release and the mean life of ACh molecules. Based on this model, the effects of tacrine, Hup-A, and E2020 were assessed by measuring the changes induced in the amplitude and duration of MEPP due to that their facilitation of cholinergic transmission resulted from the ability of anticholinesterase to prolong the postsynaptic action of ACh[11,12]. In this study, tacrine increased the amplitude and the

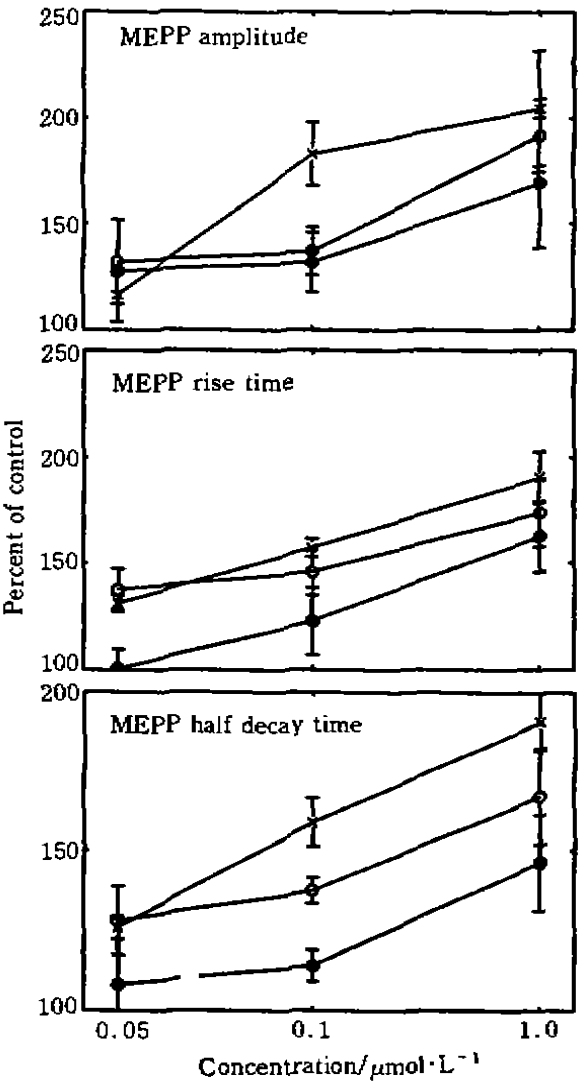


**Fig 1. Effects of huperzine-A (○), tacrine (●), and E2020 (×) on MEPP amplitude, rise time, and half decay time. $n = 3$ preparations, $\bar{x} \pm s$.**

time course of MEPP, which were agreed with early reports[13,14]. For Hup-A, the similar effects are observed with more increased amplitude, slower rise time and slower half decay time than those of tacrine, indicating that Hup-A can facilitate neuromuscular cholinergic transmission and has stronger anti-AChE efficacy than tacrine. The effects of E2020 on MEPP are stronger than those of Hup-A, but it exhibited less selectivity for AChE than Hup-A (unpublished data), while less specific inhibition on AChE may result in notable peripheral side effects. At present, of all the attempts at symptomatic therapy for AD based on the cholinergic hypothesis, using AChE inhibitors has been the most encouraging. The more potent effect of Hup-A on cholinergic transmission observed in this study and its more selective inhibition on AChE, further support the notion that Hup-A is a promising candidate in alleviating symptoms of Alzheimer's disease.

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299-301

## 石杉碱甲对离体小鼠神经肌肉接头递质传递的易化作用

R964
R971

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**关键词** 胆碱酯酶抑制剂；石杉碱甲；他克林；E2020；神经肌肉接头；膈；膈神经；电生理学

**目的**：研究石杉碱甲在小鼠神经肌肉接头处对递质传递的作用。**方法**：用胞内记录的方法在小鼠的膈神经肌肉标本上研究了石杉碱甲(Hup-A)，他克林(tacrine)和 E2020 对自发释放的小终板电位的作用。**结果**：Hup-A，tacrine 和 E2020 均可增强小终板电位的振幅、上升相和半下降相，有剂量依赖关系，其抑制强度为 E2020 > Hup-A > tacrine。**结论**：Hup-A 在胆碱能突触处对乙酰胆碱酯酶的抑制作用强于 tacrine。