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Original Research

Learning deficits induced by 4 belladonna alkaloids are preferentially attenuated by tacrine

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KEY WORDS tacrine; scopolamine; anisodine; atropine; anisodamine; belladonna alkaloids; avoidance-learning; memory; motor activity

ABSTRACT

AIM: To examine the antagonism of tacrine on the amnesic effects of scopolamine (Sco), anisodine (AT_3), atropine (Atr), and anisodamine (Ani). METHODS: Cognitive functions and locomotor activities were determined using two sessions of step-through and open-field tests, respectively. Mice were injected with one of the belladonna alkaloids (0.05－50 $\mu mol \cdot kg^{-1}$, ip) and tacrine (50 $\mu mol \cdot kg^{-1}$, sc) 30 min before the first session. RESULTS: Tacrine completely blocked the avoidance-learning deficit caused by Sco 0.5 $\mu mol \cdot kg^{-1}$, AT_3 and Atr 5 $\mu mol \cdot kg^{-1}$, or Ani 50 $\mu mol \cdot kg^{-1}$. But tacrine partly antagonized the learning deficit induced by Sco 5－50 $\mu mol \cdot kg^{-1}$ or Atr and AT_3 50 $\mu mol \cdot kg^{-1}$. The avoidance-memory deficit caused by Sco 0.05－5 $\mu mol \cdot kg^{-1}$ or Atr 5 $\mu mol \cdot kg^{-1}$ was completely or partly attenuated by tacrine, which did not antagonize the memory deficit elicited by Sco and Atr 50 $\mu mol \cdot kg^{-1}$, AT_3 5 and 50 $\mu mol \cdot kg^{-1}$, and Ani 50 $\mu mol \cdot kg^{-1}$. During the acquisition, the locomotor activity of the mice was inhibited by tacrine. This reduction was completely antagonized by Sco 0.5－50 $\mu mol \cdot kg^{-1}$, AT_3 5－50 $\mu mol \cdot kg^{-1}$, Atr 5－50 $\mu mol \cdot kg^{-1}$, and only partly antagonized by AT_3 and Atr 0.5 $\mu mol \cdot kg^{-1}$ or Ani 50 $\mu mol \cdot kg^{-1}$. CONCLUSION: Compared with the avoidance-memory deficit, the avoidance-learning deficit caused by belladonna alkaloids is more preferentially attenuated by tacrine.

INTRODUCTION

Cholinergic dysfunction in the brain has been observed in the dementia caused by Alzheimer disease (AD)[1,2]. Tacrine, a reversible non-competitive inhibitor of cholinesterase[3], has been used for the medical treatment of AD and extensively studied in experimental animals. A number of studies showed that tacrine could improve the cognitive functions of AD patients[4,5] and attenuate the amnesic effects of scopolamine (Sco) in animals[6,7]. Sco is a muscarinic cholinergic receptor antagonist and has been shown to cause cognitive deficits similar to those found in patients with AD. Consequently, the amnesic model induced by Sco is most widely used in the study of drugs for improving cognitive functions in AD patients.

The belladonna alkaloids atropine (Atr) and Sco are found chiefly in the plant nightshade and the shrubs *hyoscyamus niger* and *Scopola carmiolica*, respectively. Anisodine (AT_3) and anisodamine (Ani) were originally discovered in a traditional medicinal herb *Scopolia tangutica* in Tibet, China. Ani has an OH group at C_7 in the structure of Atr and AT_3 has an OH at the ester portion in Sco (Fig 1).

These alkaloids can block the muscarinic cholinergic actions of acetylcholine in the central nervous system and peripheral tissues. If the muscarinic receptors in the brain are blocked by these alkaloids, learning and memory will be impaired. The amnesic model induced by Sco is well known. But Atr and AT_3-induced cognitive deficits are also included in the amnesic model[8,9]. However, because of the inhibition of blood coagulation and thrombotic formation as well as the reduction of intracel-

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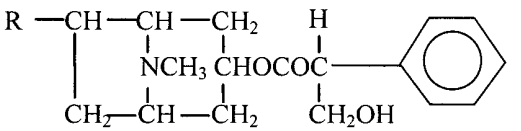
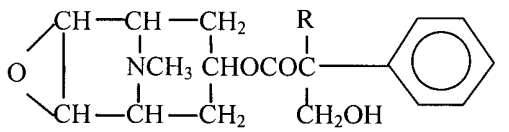
lular Ca^{2+} accumulation caused by ischemia, Ani might improve the cognitive deficits induced by brain damage[10,11].

The amnesic effects of the 4 belladonna alkaloids were compared in our previous work[12]. The present study examined the attenuation of belladonna alkaloid-induced cognitive deficits by tacrine.



R = H Atropine

OH Anisodamine



R = H Scopolamine

OH Anisodine

Fig 1. Chemical structures of 4 belladonna alkaloids.

MATERIALS AND METHODS

Mice Mice (♂, weighing 21 - 25 g) from the Kunming strain were obtained from the Center of Laboratory Animals, Institute of Epidemiology and Microbiology, Chinese Academy of Prevention Medicine. The mice were kept on a 12∶12 h light/dark cycle (lights on 07∶00) at 22 - 23 ℃. The mice were housed 8 to a cage with continuous access to food and water and maintained at laboratory conditions at least 4 d prior to the behavioral experiments.

Drugs Tacrine was purchased from Sigma Chemical Co, USA. Sco was obtained from E Merck, Darmstadt, Germany. Atr was purchased from China Medicinal Co, Beijing, China. AT_3 was a generous gift from the Institute of Materia Medica, Chinese Academy of Medical Sciences. Ani was purchased from Tianqing Pharmaceutical Factory, Tianqing, China. All drugs were dissolved in the normal saline and injected at a volume of 10 mL·kg^{-1}. Tacrine was injected sc plus vehicle ip. Belladonna alkaloids were injected ip plus vehicle or tacrine sc. In the control mice, the vehicle at 10 mL/kg was given sc and ip.

Behavioral experiments Experimentally naive male mice were individually placed in an open-field chamber for 3 min. This chamber (32 cm × 21 cm × 17 cm) was made of Perspex. The mice were not handled prior to exposure to the situation. The locomotor activity (walking about) counts throughout the 3-min test were monitored by an activity meter, as previously described[12]. The recall (the second) session was given 24 h after the acquisition (the first) session. The open-field memory was indicated by the decreased activity in the recall session compared with the acquisition session. After the open-field test, the mice were made to perform the step-through task. The apparatus consisted of a Perspex box with 2 chambers∶Only the dark chamber, not the light (safe) one, had a grid floor that could be electrified. Each mouse was first placed in the safe room. When it stepped into the dark room, the mouse received a shock punishment (40 V, 50 Hz). The latency period before the first footshock and the number of shock punishments within 3 min were measured in the training session. The retention session was conduced 24 h after the training. If a mouse did not cross into the shock room within 3 min, the retention session was ended and a score of 180 s was assigned.

Behavioral experiments were conducted between 9∶00 and 13∶00. Drugs were administered 30 min before the first session and no drug was given before the second session.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared with ANOVA and the Dunnett's multiple comparison test.

RESULTS

Locomotor activity in the open-field In the open-field test, tacrine 50 μmol·kg^{-1} significantly reduced the locomotor activity counts [$F(1, 18) = 95.8$, $P < 0.01$]. This effect of tacrine was completely antagonized by Sco 0.5, 5, and 50 μmol·kg^{-1} [$F(4, 45) = 15.2$, $P < 0.01$], AT_3 5 and 50 μmol·kg^{-1} [$F(3, 36) = 22.4$, $P < 0.01$], Atr 5 and 50 μmol·kg^{-1} [$F(3, 36) = 9.3$, $P < 0.01$], and was also partly antagonized by Ani 50 μmol·kg^{-1} [$F(3, 36) = 7.5$, $P < 0.01$]. AT_3 5 μmol·kg^{-1} increased the locomotor activity 40 % [$F(3, 36) = 5.4$, $P < 0.01$] in the acquisition session and 39 % [$F(3, 36) = 7.3$, $P <$

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0.01] in the recall session. This effect of AT_3 was inhibited by tacrine 50 μmol·kg^{-1}. However, tacrine did not by itself influence the locomotion in the recall session. In the control mice, the activity in the recall session was reduced by about 30 % compared with the acquisition session ($P<0.01$) (Fig 2).

Footshock punishment in the step-through After the open-field test, the avoidance-response of each mouse was examined using the step-through task. In the training session, the belladonna alkaloids increased the number of shock punishments [Sco：$F(4,45)=10.6$, $P<0.01$；AT_3：$F(3,36)=12.7$, $P<0.01$；Atr：$F(3,36)=42.9$, $P<0.01$；Ani：($F(3,36)=19.4$, $P<0.01$]. The minimal effective doses for increasing the footshocks were 0.5, 5, 5, and 50 μmol·kg^{-1} for Sco, AT_3, Atr, and Ani, respectively. Tacrine 50 μmol·kg^{-1} did not by itself affect the number of the shock punishments. But this dose of tacrine could completely suppress the increased number of footshocks caused by Sco 0.5 μmol·kg^{-1} [$F(1,18)=4.9$, $P<0.05$], Atr 5 μmol·kg^{-1} ($F(1,18)=27.1$, $P<0.01$], and Ani50 μmol·kg^{-1} [$F(1,18)=20.3$, $P<0.01$]. However, tacrine partly reduced the number of footshocks in the mice receiving Sco 5 μmol·kg^{-1} [$F(1,18)=5.2$, $P<0.05$] and 50 μmol·kg^{-1} [$F(1,18)=4.6$, $P<0.05$], AT_3 50 μmol·kg^{-1} [$F(1,18)=8.24$, $P<0.05$], or Atr 50 μmol·kg^{-1} [$F(1,18)=12.6$, $P<0.01$] (Fig 3).

Retention latency in the step-through The retention session was carried out 24 h after the training

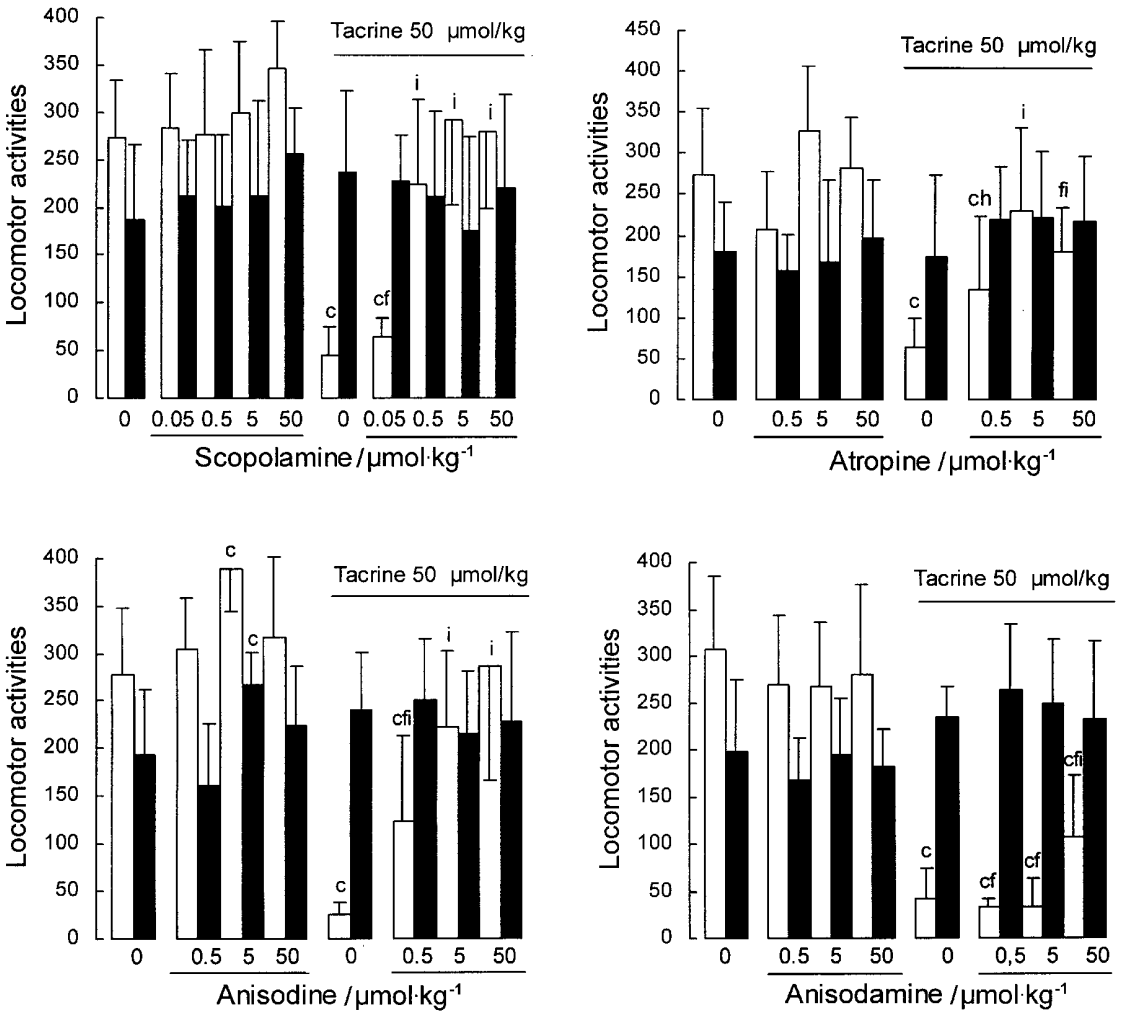

Fig 2. Attenuation of tacrine-induced depression in locomotor activity in mice by belladonna alkaloids. Drugs were injected sc for tacrine and ip for belladonna alkaloids 30 min prior to the acquisition session (□). No drug was given before the recall session (■). Recall session was carried out 24 h after the acquisition session. $n=10$ mice, $\bar{x}\pm s$. $^cP<0.01$ *vs* control (0). $^fP<0.01$ *vs* belladonna alkaloids. $^hP<0.05$, $^iP<0.01$ *vs* tacrine.

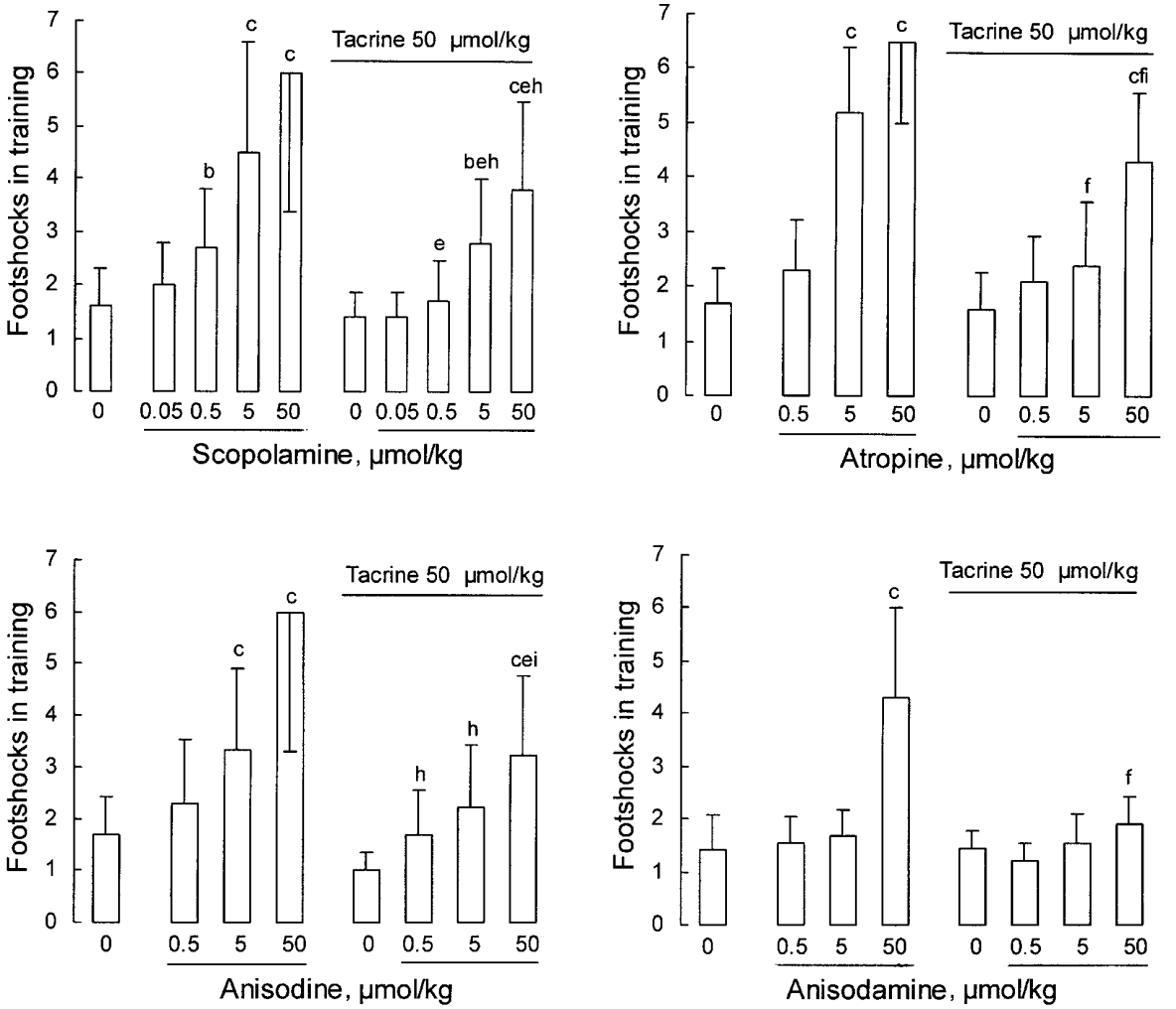

Fig 3. Attenuation of belladonna alkaloids-induced avoidance-learning deficit in mice by tacrine. This experiment was carried out after the open-field test during the acquisition session. During the training session the latencies are not different between medicine and vehicle (data not shown). $n = 10$ mice, $\bar{x} \pm s$. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control (0). $^{e}P<0.05$, $^{f}P<0.01$ *vs* belladonna alkaloids. $^{h}P<0.05$, $^{i}P<0.01$ *vs* tacrine.

session. Results showed that injection of belladonna alkaloids shortened the retention latency of receiving the shock punishment [Sco: $F(4,45) = 30.4$, $P<0.01$; AT_3: $F(3,36) = 10.1$, $P<0.01$; Atr: $F(3,36) = 29.2$, $P<0.01$; Ani: $F(3,36) = 40.3$, $P<0.01$]. The lowest doses for decreasing the retention latency were 0.05, 5, 5, and 50 $\mu mol \cdot kg^{-1}$ for Sco, AT_3, Atr, and Ani, respectively. Tacrine 50 $\mu mol \cdot kg^{-1}$ given before the training session did not alter the retention latency. Tacrine at this dose prolonged the shortened retention latency caused by Sco 5 $\mu mol \cdot kg^{-1}$ [$F(1,18) = 7.7$, $P<0.05$] and Atr 5 $\mu mol \cdot kg^{-1}$ [$F(1,18) = 8.9$, $P<0.01$]. However, tacrine did not markedly prolong the shortened retention latency by Sco and Atr 50 $\mu mol \cdot kg^{-1}$, AT_3 5 or 50 $\mu mol \cdot kg^{-1}$, and Ani 50 $\mu mol \cdot kg^{-1}$ ($P>0.05$) (Fig 4).

DISCUSSION

The passive avoidance-response in the step-through task is widely used to examine the cognitive function of animals. Belladonna alkaloids given 30 min before the training session resulted in dose-dependent increase in the number of shock punishments within 3 min. These results indicate that these alkaloids could impair the learning ability of mice. When 50 $\mu mol \cdot kg^{-1}$ tacrine given in

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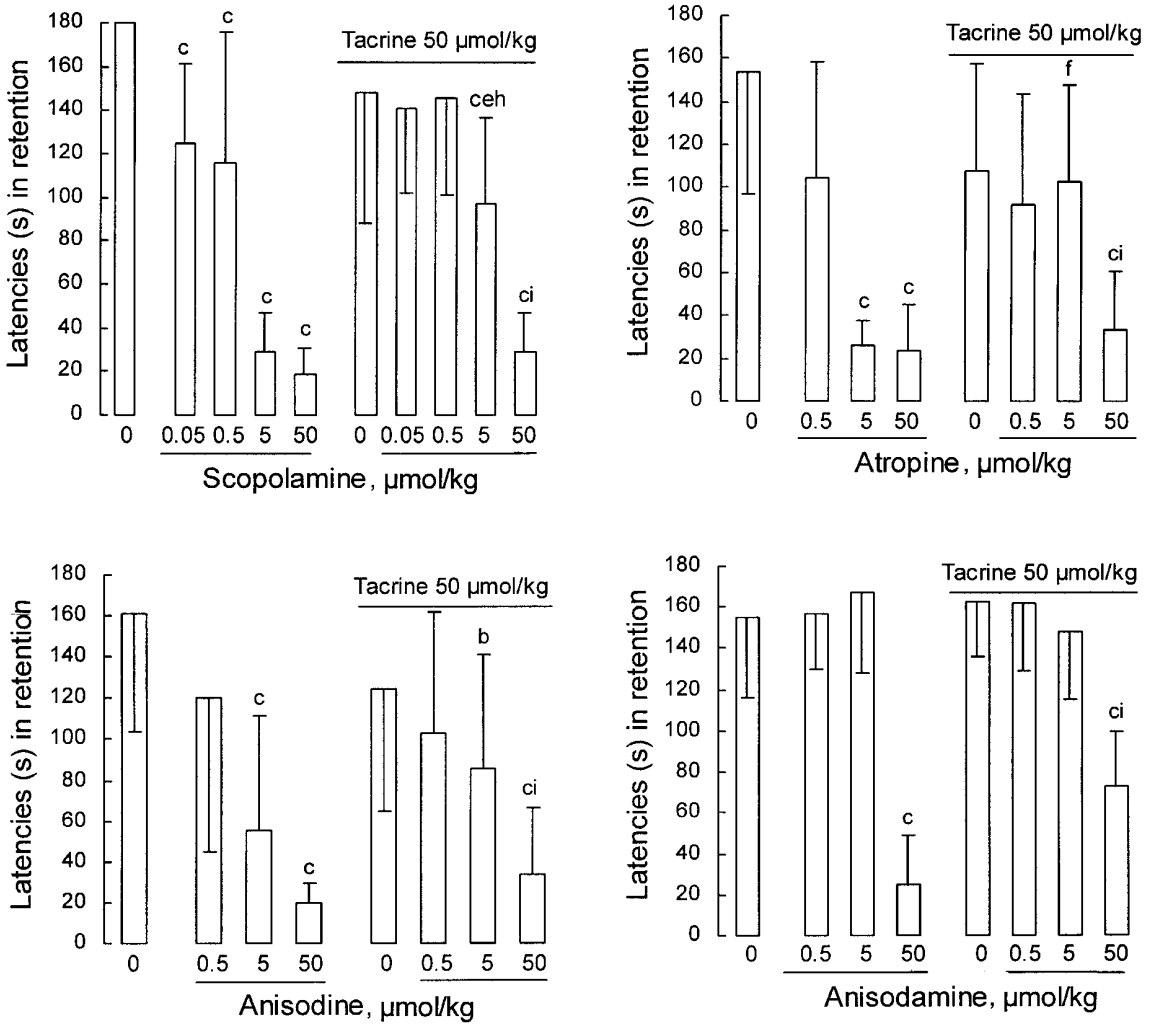

Fig 4. Attenuation of belladonna alkaloids-induced avoidance-memory deficit in mice by tacrine. This experiment was carried out after the open-field test during the recall session. $n = 10$ mice, $\bar{x} \pm s$. $^{b}P<0.05$, $^{c}P<0.01$ *vs* control (0). $^{e}P<0.05$, $^{f}P<0.01$ *vs* belladonna alkaloids. $^{h}P<0.05$, $^{i}P<0.01$ *vs* tacrine.

conjunction with these alkaloids, the dose-effect curve for increasing footshocks was shifted to the right, but the spontaneous locomotor activity of the mice was not affected compared with belladonna alkaloids alone, except in conjunction with Ani. This means that tacrine has a specific antagonism on the belladonna alkaloids-induced learning deficit. The shortened retention latency caused only by Sco and Atr, but not by AT_3 and Ani, was prolonged by tacrine. This means that tacrine could not attenuate the AT_3- and Ani-induced avoidance-memory deficit. But tacrine could reduce the AT_3- and Ani-induced learning deficit. Perhaps the memory deficit caused by AT_3 and Ani was not related to the cholinergic system in the brain or the mechanisms of memory impairment of AT_3 and Ani are different from Sco and Atr.

The present and previous studies[8,13] indicate that Sco at the doses that did not impair the passive avoidance-response learning (eg, did not increase the number of footshocks in the training session) blocked the avoidance-response memory (eg, decreased the retention latencies) in the step-through task. And tacrine could block the learning deficit elicited by 50 μmol·kg^{-1} of belladonna alkaloids, but could not block the memory deficit caused by the same doses of these alkaloids. We, therefore, propose that muscarinic cho-

linergic antagonists mainly inhibit the memory process, but that acetylcholinesterase inhibitor mainly antagonizes the learning deficit of the amnesic effect of muscarinic cholinergic antagonists. This also means that the mechanisms in the cholinergic nervous system for maintaining learning and memory are different.

It has been shown that tacrine (10 mg/kg, *po*) increased the locomotion in rats[14]. In the present study we found that tacrine at the dose of 50 μmol·kg^{-1} (11.735 mg·kg^{-1}, sc) significantly suppressed the spontaneous movement of mice. The reduced locomotion can be attenuated by the 4 belladonna alkaloids. Sco and Ani respectively exhibited the highest and lowest efficacy of antagonism on depression in locomotor activity induced by tacrine among these alkaloids. This might be related to the different concentration of these belladonna alkaloids in the brain or to their affinity with muscarinic receptors.

In the control mice, locomotion in the recall session was decreased compared with the acquisition session ($P < 0.01$). This was probably resulted from the memory of the mice on the open-field chamber[15]. AT_3 (5 μmol·kg^{-1}) not only increased the locomotor activity during the acquisition session, but also inhibited the open-field memory (eg, increased the activity in the recall session), which could be attenuated by tacrine 50 μmol·kg^{-1}. This indicated that tacrine could also block the impairment of open-field memory caused by AT_3. In the previous work, Sco and Atr injected 15 min before the acquisition session increased the locomotor activity and blocked the open-field memory[12]. In the present work, however, these two drugs given 30 min before the acquisition the increase in activity and impairment of the open-field memory were not find. This may relate to the long period from the drugs treatment to the open-field test and lead to a lower drug concentration in the mouse's body.

In summary, in this study we observed that the Sco- and Atr-induced learning and memory deficits were significantly attenuated by tacrine. But the attenuation of learning impairment was more powerful than that of memory impairment. Tacrine could only antagonize the learning deficit, and not memory impairment caused by AT_3 and Ani. Tacrine-induced depression in locomotor activity was completely antagonized by Sco, AT_3, or Atr and was partly antagonized by Ani.

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他克林优先拮抗 4 种莨菪类生物碱引起的小鼠学习障碍

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关键词　他克林；东莨菪碱；樟柳碱；阿托品；山莨菪碱；莨菪类生物碱；回避学习；记忆；运动活动

目的：比较他克林(tacrine，50 $\mu mol \cdot kg^{-1}$，sc)拮抗莨菪类生物碱(0.05－50 $\mu mol \cdot kg^{-1}$，ip)所致学习记忆障碍．　方法：用两次性开阔和回避反应研究，小鼠于第一次实验前 30 min 给药．　结果：他克林对东莨菪碱(Sco) 0.05－50 $\mu mol \cdot kg^{-1}$ 或阿托品(Atr) 5－50 $\mu mol \cdot kg^{-1}$ 引起的学习和记忆障碍均有拮抗作用，以拮抗学习障碍最明显．　他克林能改善樟柳碱(AT_3)和山莨菪碱(Ani) 5－50 $\mu mol \cdot kg^{-1}$ 所致学习损伤，但不能拮抗其记忆损伤．Sco 0.5－50 $\mu mol \cdot kg^{-1}$，AT_3 和 Atr 5－50 $\mu mol \cdot kg^{-1}$ 完全拮抗，Ani 50 $\mu mol \cdot kg^{-1}$ 部分拮抗他克林引起的自主活动减少．　结论：他克林优先改善莨菪类药物引起的被动回避反应学习障碍．

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