BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Mar; 19 (2): 112 - 116

Belladonna alkaloids-induced behavioral changes and amnesia on open-field and step-through in 18-, 28-, and 38-day-old mice

PAN Si-Yuan[1], HAN Yi-Fan[2], XU Qiu-Ping, LIU Ming-Yan, MA Hui-Zhen, DING Wei-Jing, ZHU Ren-Liang
(*Department of Pharmacology, Beijing University of Traditional Chinese Medicine, Beijing 100029, China*; [2]*Department of Biochemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China*)

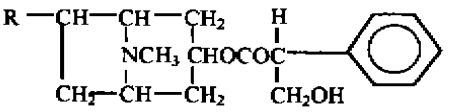
KEY WORDS animal behavior; learning; memory; brain; receptors; atropine; scopolamine; anisodine; anisodamine; belladonna alkaloids

AIM: To study the age-related changes of atropine (Atr), scopolamine (Sco), anisodine (AT_3), and anisodamine (Ani) on behaviors and memories. **METHODS**: The behaviors and memories were measured with open-field test and step-through task. M-cholinergic receptors were determined by [^{3}H] quinuclidinyl benzilate ([^{3}H]QNB). **RESULTS**: During acquisition session (d 1) the 18-, 28-, and 38-d-old mice pretreated with Atr, Sco, and AT_3 (0.02, 0.2, 2, or 20 mg·kg^{-1}, ip) in open-field test showed increase in walking counts by 26 % - 42 %, but decrease in rearing, grooming, and defecating counts for 50 % - 92 %, 67 % - 100 %, and 75 % - 100 %, respectively. On recall session (d 2) the walking and rearing behaviors in the 18- and 28-d-old mice receiving Atr, Sco, and AT_3 on d 1 were higher than those in the mice receiving saline. But a lower grooming behavior on d 2 was found in the mice receiving the drugs on d 1. On d 1 Ani 20 mg·kg^{-1} reduced the rearing behavior by 50 % in 18-d-old mice and defecation by 33 % - 36 % in 18- and 28-d-old mice. All the 4 belladonna alkaloids increased the number of avoidance-response errors and decreased the retention latencies in step-through task. B_{max} of [^{3}H]QNB binding sites in frontal cortex and hippocampus regions in the 38-d-old mice increased 7 % and 23 % *vs* in the mice of 18 d of age, respectively. **CONCLUSION**: 1) The effects of the belladonna alkaloids on behaviors and memories in adult mice were weaker than those in young mice. 2) The belladonna alkaloids-induced amnesia on passive avoidance-response in step-through was more sensitive than behavioral changes and amnesia on open-field. 3) According to the lowest effective doses which insulted the behaviors or memories in young mice, Sco was about 10, 100, and 1000 times more potent than Atr, AT_3, and Ani, respectively.

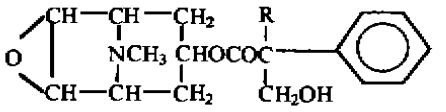
The belladonna alkaloids are widely distributed in nature, especially in the Solanaceae plants. The nightshade yields mainly the alkaloid atropine (Atr). The alkaloid scopolamine (Sco) is found chiefly in the shrub *Hyoscyamus niger* and *Scopola carniolica*. The alkaloid anisodine (AT_3) and anisodamine (Ani) were first isolated from a Tibetan traditional herb *Scopolia tangutica* in China. These alkaloids can antagonize the muscarinic actions of acetylcholine. They are therefore called antimuscarinic agents, which are widely used in medical practice and extensively studied in experimental animals. They are well known to produce memory deficits in humans[1,2] and animals[3,4] or behavioral changes[5,6]. Their therapeutic doses, especially Sco, can cause drowsiness, euphoria, fatigue, dreamless sleep, and amnesia. However, the same doses occasionally cause excitement, restlessness, hallucinations, or delirium instead. In experimental animals, the hydrolysis of phosphoinositides elicited by stimulation of cholinergic muscarinic receptors was higher in neonatal rats than in adult rats, and in hippocampus and cerebral cortex the response was higher than in cerebellum or brain stem[7].

The penetration of Ani into central nervous system (CNS) is more difficult. There was no report on the amnesia produced by Ani, and the relationship between amnesia and behavioral changes elicited by belladonna alkaloids. The purpose of the present study was to compare the behavioral and amnesic effects of the 4 belladonna alkaloids, and M-cholinergic receptors in various brain regions in immature and mature mice.

[1] Corresponding author.
Phn: 86-10-6428-6934. Fax 86-10-6421-3841.
Received 1996-11-22 Accepted 1997-10-04



R = H Atropine
OH Anisodamine



R = H Scopolamine
OH Anisodine

MATERIALS AND METHODS

Mice were 18 d (weighing 11 – 14 g)-, 28 d (weighing 21 – 24 g)-, and 38 d (weighing 31 – 34 g)-d-old ♂ mice of Kunming strain and supplied by China Academy of Traditional Chinese Medicine. They were housed 8 to a cage at 20 – 21 ℃ and light on from 7:00 to 19:00. The behavioral and memory experiments were carried out between 8:00 and 12:00.

Drugs Sco was purchased from E Merck, Darmastadt. AT_3 was from Institute of Materia Medica, Chinese Academy of Medical Sciences. Atr and Ani were bought from Beijing Pharmaceutical Factory. [^{3}H]QNB, 455.1 TBq·mol^{-1}, was purchased from Amersham, UK. Sco, Atr, AT_3, and Ani were prepared in normal saline and ip 10 mL·kg^{-1} 15 min before the acquisition session (d 1) in open-field test.

Open-field test Each mouse was tested for 3 min daily for 2 successive days in a 32 cm × 21 cm × 15 cm open-field chamber, which was totally unfamiliar. Walking, rearing, grooming, and defecation in both acquisition session (d 1) and recall session (d 2) were recorded. The rearing behavior was indicated by the standing on hindlimbs with stretched back. The numbers of walking were automatically recorded with an Activity Meter. The grooming behaviors included washing face and licking body or paws. The defecations were indicated by the number of boluses deposited during a 3-min period.

Step-through task The shock avoidance apparatus consisted of a perspex box of 2 compartments. The dark compartment was a grid floor which could be electrified. But there was not electrified in the safe room. Each mouse was put in the safe compartment. After stepped from the safe room to the dark room mouse received a footshock (50 V, 50 Hz), after which the mouse returned to the safe room and avoided entering the dark compartment. During training (d 1) test the number of avoidance-response errors indicated the times the mouse entered the dark chamber to receive footshock in a 3-min period. Retention test was given 24 h following the training test. If a mouse did not enter the dark room within 3 min, the mouse was assigned a score of 180 s.

Determination of muscarinic receptors Mice of 18 and 38 d of age were decapitated. The frontal cortex, striatum, hippocampus, and midbrain were taken in a dish on ice. Tissues from 10 mice were pooled for use at each age. The tissues were homogenized in 20 volumes of 50 mmol·L^{-1} phosphate buffer, pH 7.4, and then spun at 4 ℃ at 15 000 × g for 10 min. The pellet was resuspended and washed twice with 20 volumes of buffer to remove endogenous ligands. The final pellet was resuspended at a protein concentration of about 100 g·L^{-1} and frozen at – 20 ℃ until binding assay. The tissues in 100 – 150 μg protein/tube were incubated in 1 mL of buffer containing 6 concentrations (0.02 – 2 nmol) of [^{3}H]QNB. Parallel tubes containing atropine (1 μmol·L^{-1}) were used to determine nonspecific binding. After incubation at 37 ℃ for 1 h, the samples were filtered through glass filters with vacuum and then dried at 80 ℃. Radioactivity trapped on the filters was determined by a liquid scintillation counter (Beckman 5801) at a counting efficiency of 60 %. The specific binding was calculated as total minus nonspecific binding[8]. Protein contents were determined using Folin phenol reagent with bovine serum albumin as a standard[9].

Data analysis The affinity (as K_d) and density (as B_{max}) of [^{3}H]QNB binding sites were calculated from the Scatchard polts. All the experimental results were expressed as $\bar{x} \pm s$ and compared using the two-tailed t-test.

RESULTS

Behaviors in open-field test

Walking behavior The lowest effective doses of Atr to increase the locomotion by 24 % – 52 % on both d 1 and d 2 were 2 and 20 mg·kg^{-1} for 18- and 28-d-old mice, respectively. The minimal effective doses of Sco for increasing locomotion (26 % – 64 %) were 0.02 and 0.2 mg·kg^{-1} for 18- and 28-d-old mice, respectively. However, Atr 20 mg·kg^{-1} and Sco 0.2 mg·kg^{-1} increased the locomotion by 20 % – 27 % only on d 1, not on d 2, in the 38-d-old mice. AT_3 2 mg·kg^{-1} increased the ambulation by 23 % – 31 % on d 1 in the mice of the 3 ages. On d 2 the 28-d-old mice which receoved AT_3 2 or 20 mg·kg^{-1} still showed a higher locomotion vs control group ($P < 0.05$ or $P < 0.01$). Ani did not alter the locomotor activity in all the mice. The number of open-field ambulation in young mice were less (26 %) than those in adult mice ($P < 0.05$). The open-field ambulation in the 18- and 28-d-old mice on d 2 was reduced by 28 % – 52 % *vs* on d 1 in the control mice ($P < 0.05$ or $P < 0.01$).

Rearing behavior When the mice of 18 or 28 d

old were replaced in the open-field chamber on d 2, the rearing times were decreased 50 % – 75 % *vs* on d 1 in control group ($P < 0.01$). Mice which medicated 15 min prior to the acquisition session (d 1) in the open-field test showed the decrease (on d 1) or increase (on d 2) in rearing behavior. On d 1 the minimal effective doses of Atr, Sco, AT_3, and Ani for inhibiting the rearing behavior by 67 %, 63 %, 64 %, and 50 % in the mice of 18 d of age were 0.2, 0.02, 2, and 20 mg·kg^{-1}, respectively. The lowest doses which inhibited the rearing behavior by 42 % – 73 % on d 1 in the 28- and 38-day-old mice were Atr 2 mg·kg^{-1}, Sco 0.02 or 2 mg·kg^{-1}, and AT_3 2 mg·kg^{-1} ($P < 0.05$ or $P < 0.01$). On d 2 a higher rearing behavior was noted in the 18- and 28-day-old mice which received Atr or Sco on d 1 ($P < 0.05$ or $P < 0.01$). But Sco 20 mg·kg^{-1} reduced the rearing on d 2 in the mice of 38 d old ($P < 0.05$).

Grooming behavior The grooming behavior in 18-d-old mice was much more than that in 38-d-old mice, particularly in the recall session ($P < 0.01$). On d 2 the control mice (18- and 28-d-old) spent much more time to groom *vs* d 1 ($P < 0.05$ or $P < 0.01$). The mice pretreated with Atr, Sco or AT_3 on d 1 showed a lower behavior of washing face, licking body and paws ($P < 0.05$ or $P < 0.01$). The lowest doses of Atr for influencing the grooming behavior on d 1 were 20, 20, and 2 mg·kg^{-1} for 18-, 28-, and 38-d-old mice, respectively. But the minimal effective doses of Sco which inhibited the groom by 63 %, 80 %, and 75 % on d 1 were 0.02, 0.2, and 20 mg·kg^{-1} for 18-, 28-, and 38-d-old mice, respectively. On d 1 the lowest effective dose of AT_3 inhibiting the groom in the mice of the 3 ages was 2 mg·kg^{-1}. The 18-d-old mice received Atr 20 mg·kg^{-1}, Sco 20 mg·kg^{-1}, and AT_3 2 or 20 mg·kg^{-1} on d 1 showed a lower groom on d 2 *vs* control ($P < 0.05$ or $P < 0.01$). A lower grooming behavior on d 2 was also found in the 28-d-old mice which was administered Sco or AT_3 on d 1 ($P < 0.05$).

Defecating behavior The number of the boluses deposited on d 2 was higher than those on d 1 in all the mice ($P < 0.05$ or $P < 0.01$). The 4 drugs injected on d 1 inhibited the defecation by 60 % – 100 % on d 1, but did not alter the defecating behavior on d 2. The lowest effective dose of Atr for inhibiting defecation was 2 mg·kg^{-1} in all the mice. The minimal effective doses of Sco were 0.02, 0.2, and 2 mg·kg^{-1} for 18-, 28-, and 38-d-old mice, respectively. The effective dose of AT_3 and Ani was 20 mg·kg^{-1} in both 18- and 28-d-old mice. However, the lowest effective dose of AT_3 which reduced boluses was 2 mg·kg^{-1} in the 38-d-old mice.

Avoidance-response in step-through task

After the open-field test, mice were immediately put in the step-through task each day. The premedicated mice on d 1 showed a dose-dependent impairment of avoidance-response learning (increase in the number of footshock in training) and memory (decrease in the latencies of receiving footshock in retention). The lowest effective doses for inhibiting the avoidance-response in all the mice were Atr 0.2 mg·kg^{-1}, AT_3 2 mg·kg^{-1}, and Ani 20 mg·kg^{-1}. However, the minimal doses of Sco for disrupting the process of avoidance-response learning in the 18-, 28-, and 38-d-old mice were 0.02, 0.2, and 2 mg·kg^{-1}, respectively. But the doses of Sco which blocked the avoidance-response memory process were 0.02, 0.02, and 2 mg·kg^{-1} for the mice of 18, 28, and 38 d of age, respectively (Tab 1).

Muscarinic receptors The density of muscarinic receptors in frontal cortex and hippocampus regions in 38-d-old mice was greater than that in 18-d-old mice. There were no significant age-related differences in the [^{3}H]QNB binding sites in striatum and midbrain regions. The affinity constants of [^{3}H]QNB for M-cholinergic receptors were not different in all brain regions in both ages (Tab 2).

DISCUSSION

When placed into a novel or unfamiliar environment, mice appear several different behavioral expressions. The rank order of the potencies of belladonna alkaloids for disrupting behaviors in open-field were Sco > Atr > AT_3 > Ani. The rank order of the susceptibilities of the behaviors on the belladonna alkaloids were rear > locomotion > defecation > groom. When replaced into the open-field chamber after 24 h, the 18- and 28-d-old mice exhibited a reduced motility and rearing, but the grooming and defecation were enhanced. These results might be due to the memory

Tab 1. Minimal effective doses ($mg \cdot kg^{-1}$) of belladonna alkaloids-induced behavioral changes and amnesia in 18-, 28-, and 38-d-old mice. The behaviors and avoidance-response were tested with open-field and step-through, respectively. Drugs (0.02, 0.2, 2, or 20 $mg \cdot kg^{-1}$) were given ip 15 min before the test on d 1. On d 2 the walking and rearing behaviors in the control mice were decreased, but the grooming and defecating behaviors were increased *vs* on d 1. Mice receiving belladonna alkaloids showed increase in walking behavior (on d 1 and d 2) and rearing behavior (on d 2), but decrease in rearing (on d 1), grooming (on d 1 and d 2), and defecating (on d 1) behaviors. The inhibition of the 4 drugs on avoidance-response showed the increase in the times of footshocks in training session (d 1) and decrease in the latencies of receiving footshock in retention session (d 2).

	To induce behavioral changes								To inhibit avoidance-response	
	Walking		Rearing		Grooming		Defecating			
	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
Scopolamine										
18 d old	0.02	0.02	0.02	0.02	0.02	20	0.02	>20	0.02	0.02
28 d old	0.2	0.2	0.02	20	0.2	0.2	0.2	>20	0.2	0.02
38 d old	0.2	>20	2	20	20	>20	2	>20	2	2
Atropine										
18 d old	2	2	0.2	20	20	20	2	>20	0.2	0.2
28 d old	20	20	2	20	20	>20	2	>20	0.2	0.2
38 d old	20	>20	2	>20	2	>20	2	>20	0.2	0.2
Anisodine										
18 d old	2	>20	2	>20	2	2	20	>20	2	2
28 d old	2	2	2	>20	2	2	20	>20	2	2
38 d old	2	>20	2	>20	2	>20	2	>20	2	2
Anisodamine										
18 d old	>20	>20	20	>20	>20	>20	20	>20	20	20
28 d old	>20	>20	>20	>20	>20	>20	20	>20	20	20
38 d old	>20	>20	>20	>20	>20	>20	>20	>20	20	20

Tab 2. [^{3}H]QNB binding sites in brain regions in 18- and 38-d-old mice. $n = 10$ mice, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* 18-d-old mice.

Brain regins	[^{3}H]QNB binding parameters	
	B_{max}, pmol/g protein	K_d, $nmol \cdot L^{-1}$
Frontal cortex		
18 d old	1 025 ± 26	0.133 ± 0.010
38 d old	1 108 ± 63^b	0.154 ± 0.024^a
Corpus striatum		
18 d old	1 489 ± 133	0.149 ± 0.045
38 d old	1 332 ± 177^a	0.138 ± 0.037^a
Hippocampus		
18 d old	410 ± 8	0.053 ± 0.009
38 d old	539 ± 30^c	0.057 ± 0.010^a
Midbrain		
18 d old	330 ± 25	0.080 ± 0.026
38 d old	353 ± 46^a	0.061 ± 0.020^a

and/or adaptation of mice on this chamber. This memory was easy acquisition and decline, and was not easily blocked by drugs *vs* passive-avoidance memory in step-through[10]. During recall session (d 2) the open-field behaviors in the mice pretreated with Atr, Sco, or AT_3 on d 1 were similar to those on d 1 in control mice. These indicated that the memory or adaptation of mice on open-field chamber was blocked by the belladonna alkaloids. Atr, Sco, and AT_3 at the doses that completely disrupted the memory in step-through task affected all the behaviors in open-field test. But the dose of Ani which impaired the avoidance-response memory did not influence the open-field behaviors, excepting the defecation, and memory. Thence the amnesic or Alzheimer's disease model elicited by Ani in step-through task may be better than that by other belladonna alkaloids, especially Sco.

The density of cholinergic muscarinic receptors was increased in brain during development and reached adult levels in the rat about postnatal 30 d[11]. And the receptors in the medulla were present in higher numbers at birth and reached adult values more rapidly than those in cerebral cortex[12]. Our results indicated that the number of [^{3}H]QNB binding sites in frontal cortex and hippocampus in the mice at 38 d of age were

greater than those in the mice at 18 d of age. These data suggested that the rate of the development of the maturation of M-cholinergic receptor density was different in different brain areas. Because the central anticholinergic effects of muscarinic antagonists are related to the number of muscarinic receptors in brain[13,14], and the cholinergic transmission in cortex and hippocampus aeras plays an essential role in memory processes[15]. In the present study, the lower muscarinic receptor densities in frontal cortex and hippocampus in immature mice might, at least in part, explain their high response to belladonna alkaloids-induced impairment of open-field and avoidance-response memory, and open-field behaviors.

In summary, the belladonna alkaloids induced behavioral changes and amnesia in young mice were more sensitive than those in adult mice. The minimal effective doses required to disrupt behaviors and open-field memory were larger than avoidance-response memory. The lowest doses of these drugs for disrupting open-field behaviors were lower than those for blocking open-field memory. [^{3}H]QNB binding sites in cortex and hippocampus in the mice at 18 d of age were lower than those in the mice at 38 d of age.

REFERENCES

1 Rusted JM, Warburton DM. The effects of scopolamine on working memory in healthy young volunteers. Psychopharmacology 1988; 96: 145 – 52.

2 Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl SM. Modelling dementia: effects of scopolamine on memory and attention. Neuropsychologia 1988; 26: 685 – 700.

3 Diaz del Guante MA, Cruz-Morales SE, Prado-Alcala RA. Time-dependent effects of cholinergic blockade of the striatum on memory. Neurosci Lett 1991; 122: 79 – 82.

4 Buresova O, Bolhuis JJ, Bures J. Differential effects of cholinergic blockade on performance of rats in the water tank navigation task and in a radial water maze. Behav Neurosci 1986; 100: 476 – 82.

5 Bymaster FP, Heath I, Hendrix JC, Shannon HE. Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. J Pharmacol Exp Ther 1993; 267: 16 – 24.

6 Pan SY. Circadian effects of scopolamine on memory, exploratory-behavior, and muscarinic receptors in mouse brain. Acta Pharmacol Sin 1992; 13: 323 – 6.

7 Balduini W, Murphy SD, Costa LG. Characterization of cholinergic muscarinic receptor-stimulated phosphoinositide metabolism in brain from immature rats. J Pharmacol Exp Ther 1990; 253: 573 – 9.

8 Pan SY. Acetylcholine contents and muscarinic receptor levels in frontal cortex, corpus striatum, and hippocampus of reserpinized rats and mice. Acta Pharmacol Sin 1992; 13: 223 – 6.

9 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265 – 75.

10 Pan SY. Features of memory on novel situation and avoidance response: evidence from comparisons between open-field behavior and step-through task. Acta Pharmacol Sin 1995; 16: 125 – 9.

11 Balduini W, Murphy SD, Costa LG. Developmental changes in muscarinic receptor-stimulated phosphoinositide metabolism in rat brain. J Pharmacol Exp Ther 1987; 241: 421 – 7.

12 Coyle JT, Yamamura HI. Neurochemical aspects of the ontogenesis of cholinergic neurons in the rat brain. Brain Res 1976; 118: 429 – 40.

13 Sunderland T, Tariot PN, Newhouse PA. Differential responsivity of mood, behavior, and cognition to cholinergic agents in elderly neuropsychiatric populations. Brain Res Rev 1988; 13: 371 – 89.

14 Molchan SE, Martinez RA, Hill JL, Weingartner HJ, Thompson K, Vitiello B, *et al*. Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. Brain Res Rev 1992; 17: 215 – 26.

15 Alkon DL, Amaral DG, Bear MF, Black J, Carew TJ, Cohen NJ, *et al*. Learning and memory. Brain Res Rev 1991; 16: 193 – 220.

15

112 – 116

莨菪类生物碱对 18, 28 及 38 日龄小鼠的行为和记忆障碍作用

潘思源[1], 韩怡凡[2], 徐秋萍, 刘名彦, 马慧珍, 丁委静, 朱仁良

(北京中医药大学药理教研室, 北京 100029, 中国;
[2]香港科技大学生物化学系、香港九龙清水湾, 香港)

R971.72

关键词 动物行为; 学习; 记忆; 脑; 受体; 阿托品; 东莨菪碱; 樟柳碱; 山莨菪碱; 莨菪类生物碱

目的: 比较阿托品(Atr), 东莨菪碱(Sco), 樟柳碱(AT_3)和山莨菪碱(Ani)对小鼠行为及记忆损伤作用. **方法**: 行为和记忆实验用开阔和回避反应法. 脑 M 受体用[^{3}H]QNB 测定. **结果**: Atr, Sco 和 AT_3 增加小鼠走动行为 26 % – 42 %, 降低站立, 修饰, 排便行为 50 % – 100 %, 并抑制开阔记忆. 4 个药物均能妨碍回避反应. 小鼠 18 日龄额叶皮层和海马[^{3}H]QNB结合位点数少于 38 日龄 7 % – 23 %. **结论**: 1) 莨菪类生物碱对小鼠行为和记忆障碍的作用随其日龄增加而减弱. 2) Sco 对幼年小鼠的行为及记忆障碍作用的最小有效量分别是 Atr, AT_3 和 Ani 的 1/10, 1/100 和 1/1000.