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Circadian effects of scopolamine on memory, exploratory behavior, and muscarinic receptors in mouse brain

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ABSTRACT Mice were maintained at light-dark cycle with lights on from 05:00-19:00 for 10 d in laboratory. The study was performed at 07:00-09:00, 15:00-17:00, and 21:00-23:00 in June and July. Scopolamine (Scop, 0.1 and 0.4 mg · kg^{-1}, ip) was injected 15 min prior to training or the first exploratory test. The amnesic effects of Scop showed hyperresponses at 07:00-09:00 and 15:00-17:00, and hyporesponses at 21:00-23:00 using step-through and step-down tasks. The circadian effects of Scop on exploratory behavior were consistent with the findings above mentioned. The numbers of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding sites in the temporal cortex and hippocampus were more at 08:00 and 16:00 than those at 22:00. However, muscarinic receptor levels in the striatum were lower at 08:00 than those at 22:00. These results indicated that the effects of Scop on memory, behavior in a novel environment, and muscarinic receptors in brain regions showed circadian changes in mice.

KEY WORDS scopolamine; quinuclidinyl benzilate; memory; exploratory behavior; muscarinic receptors; temporal lobe; corpus striatum; hippocampus; circadian rhythm

The status of cholinergic neurotransmitter, enzymes, and receptors in mammalian brain showed a diurnal oscillation[1-3]. As a rule, the number of free (unoccupied by endogenous agonist) muscarinic (M) receptors was highest during the light period when cholinergic neurotransmission was least active and lowest during the dark period when rats and mice were most active. In the night a minimal affinity of carbachol, an M receptor agonist, to rat brain receptors was observed. The present work attempted to test whether the diurnal changes of M receptors influenced the effects of Scop, an M receptor antagonist, on memory and behavior in mice.

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MATERIALS AND METHODS

Mice of Kunming strain, ♂, weighing 24 ± *s* 3 g, outbred from Chinese Academy of Traditional Chinese Medicine (Beijing), were maintained in a light-dark environment (light on from 05:00-19:00) at 25-29℃ in 1991 Jun and Jul.

[^{3}H]QNB, 251.4 TBq · mol^{-1}, and Scop were purchased from Amersham and Merck, respectively.

Step-through and step-down tests[4] In step-through task the latencies and the number of memory errors during 5 min were recorded in retention test. If a mouse did not enter the dark compartment within 300 s the retention test was terminated and the mouse was assigned a retention score of 300 and a zero errors. In the step-down task the mouse was placed on a platform for 3 min, then received foot electric shock (36 V) for 5 min. Retention test was given 24 h following the training. The errors indicated the number of entrances into the dark compartment in step-through or received a foot shock from the grid in step-down.

Exploratory behavior test In this study each mouse was tested for 5 min / d for 2 successive days in a 32 × 21 × 15 cm^3 box which was new to the mice. The locomotor activity was automatically measured with an Activity Meter (MK-ANIMEX, Muromachi Kikai Co. Tokyo) and the defecation was quantified by counting the number of boluses dropped.

M receeptor binding test Brain homogenates were prepared[5]. The protein contents were determined by chromatometry[6]. The brain homogenates, 150 μg protein / tube, were incubated with [^{3}H]QNB 0.02-2 nmol · L^{-1} for 60 min at 37 ℃ in 1 ml of phosphate buffer 50 mmol · L^{-1} at pH 7.4. The specitic binding of [^{3}H]QNB to M receptors was defined in the presence or absence of atropine 2 μmol · L^{-1}. The incubations were terminated by filtration through glassfiber filters. Isolated membranes were

Tab 1. Diurnal changes of amnesia induced by ip Scop in mice. Scop or saline was given 15 min before training test. Retention test was performed after 24 h. $n=12$. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **saline.** $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* **07:00–09:00 and / or 15:00–17:00.**

	Dose / mg · kg^{-1}	Latencies / s		Memory errors / times	
		Step–through	Step–down	Step–through	Step–down
07:00–09:00					
Saline	–	243 ± 97	249 ± 67	0.3 ± 0.5	0.8 ± 1.0
Scop	0.1	97 ± 112**	47 ± 64***	1.5 ± 1.1***	2.1 ± 1.3***
Scop	0.4	58 ± 104***	30 ± 44***	2.5 ± 1.3***	3.3 ± 2.1***
15:00–17:00					
Saline	–	281 ± 52	273 ± 48	0.3 ± 0.5	0.4 ± 0.7
Scop	0.1	106 ± 109**	34 ± 46***	2.1 ± 1.5**	2.2 ± 1.6***
Scop	0.4	77 ± 87**	20 ± 31***	2.2 ± 1.9**	2.6 ± 1.1***
21:00–23:00					
Saline	–	300 ± 0^{++}	300 ± 0^{++}	0 ± 0^{+}	0 ± 0^{+}
Scop	0.1	234 ± 91^{**+++}	266 ± 67^{+++}	0.5 ± 1.2^{*++}	0.5 ± 0.7^{+++}
Scop	0.4	154 ± 117^{***-}	137 ± 113^{***+-}	1.8 ± 2.7^{**+}	1.5 ± 1.4^{***-}

washed with 3 × 3 ml of ice–cold buffer and then dried at 80℃. The bound radioactivity of membranes was counted by liquid scintillation spectrometry.

RESULTS

Circadian changes in Scop–induced amnesia Scop (0.1 or 0.4 mg · kg^{-1}) was injected ip at 06:45, 14:45, and 20:45. Greater amnesic effects of Scop on mice occurred during 07:00–09:00 and 15:00–17:00 than those during 21:00–23:00. (Tab 1).

Circadian changes in Scop–induced exploratory behavior Scop was injected ip 15 min before the first session. Scop significantly increased the locomotor activity and reduced the defecation and urination during 07:00–09:00 and 15:00–17:00. But these effects of Scop (0.1 mg · kg^{-1}) on the exploratory behavior were not seen during 21:00–23:00 on d 1 and d 2. (Tab 2)

Tab 2. Diurnal changes of exploratory behavior induced by Scop in mice. Scop or saline (control) was injected ip 15 min before the test on d 1. $n=13$. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.** $^{+}P>0.05$, $^{++}P<0.05$, $^{+++}P<0.01$ *vs* **07:00–09:00 and / or 15:00–17:00.**

	Dose / mg · kg^{-1}	On the first day		On the second day	
		Activity	Defecation	Activity	Defecation
07:00–09:00					
Control	–	469 ± 89	3.1 ± 2.0	259 ± 108	4.1 ± 2.0
Scop	0.1	632 ± 79**	1.0 ± 1.1***	526 ± 71***	4.3 ± 2.3*
Scop	0.4	660 ± 58**	0.7 ± 1.2***	412 ± 160**	4.0 ± 2.0*
15:00–17:00					
Control	–	531 ± 80	2.7 ± 2.3	324 ± 156	4.9 ± 1.9
Scop	0.1	511 ± 138*	0.9 ± 1.3**	265 ± 119*	3.4 ± 2.6*
Scop	0.4	649 ± 114^{**+}	0.4 ± 0.8***	465 ± 131**	3.8 ± 2.3*
21:00–23:00					
Control	–	566 ± 59^{++}	3.9 ± 2.3^{-}	511 ± 76^{++}	5 ± 3^{+}
Scop	0.1	516 ± 124^{*-}	3.0 ± 2.0^{*-++}	458 ± 95^{*-}	6 ± 3^{*++}
Scop	0.4	528 ± 146^{*-}	0.3 ± 0.6^{***+}	492 ± 78^{*+}	5 ± 3^{*+}

Circadian changes in M receptors of brain regions Mice were killed at 08:00, 16:00, and 22:00. The temporal cortex, striatum, and hippocampus were excised on ice. Binding assay and Scatchard analysis showed that the number of M receptors in the brain regions varied with the time of a day. Binding was maximal at 08:00 and minimal at 22:00 in the temporal cortex and hippocampus. In the striatum, however, the receptor levels at 08:00 were lower than those at 16:00 and 22:00. The affinity of [^{3}H]QNB to M receptor exhibited no diurnal variation in the hippocampus but increased in temporal cortex during the light period. (Tab 3).

Tab 3. Diurnal changes of muscarinic receptors in mouse brain. $n=3$, $\bar{x}\pm s$, $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **08:00 and / or 16:00.**

Brain region	[^{3}H]QNB binding parameters B_{max}, fmol / mg protein	K_d, nmol · L^{-1}
08:00		
Temporal cortex	603 ± 31	0.150 ± 0.005
Striatum	466 ± 43	0.097 ± 0.015
Hippocampus	467 ± 14	0.145 ± 0.009
16:00		
Temporal cortex	522 ± 14**	0.158 ± 0.026*
Striatum	567 ± 31**	0.176 ± 0.010***
Hippocampus	448 ± 8*	0.159 ± 0.021*
22:00		
Temporal cortex	504 ± 22***	0.27 ± 0.05**
Striatum	562 ± 30***	0.19 ± 0.04***
Hippocampus	382 ± 46**	0.147 ± 0.026*

DISCUSSION

When exposed to an unfamiliar place animals expressed exploratory behaviors including moving about, standing, grooming and defecation that reflected the emotional state[7,8]. The exploratory activity often tended to decrease on repeated testing. This was generally regarded as adaptation to the test situation. However, in mice having received Scop the augmented activity could still be seen at the d 2 test. This suggested that Scop inhibited the mouse adaptive processes to a novel chamber as it impaired the learning and memory processes of the mouse.

Generally, the effective degree of cholinergic blocker on central nervous system depends on the numbers of brain cholinergic receptors. When the number of receptor increases or decreases the effects of cholinergic blockade are also enhanced or lessened correspondingly. Although in this paper the binding assay showed that the diurnal changes of M receptors in the temporal cortex and hippocampus could explain the circadian rhythm of Scop on memory and behavior, other factors should also be considered. For example, Scop reduced glucose utilization in several areas of the cerebral cortex[9] and cerebral glucose utilization was related to mental activity[10]. In addition, when injected into the striatum Scop caused amnesia[11], but from the present data, the M receptors in this region was fewest at 08:00. However, at this o'clock the action of Scop was most pronounced.

As the laboratory conditions were limited, the mice had to be maintained in natural light–dark cycle and temperature in summer. The light phase of the cycle was longer than the dark one and the room temperature was quite high (26–29℃ at daytime and 25–27℃ at night).

REFERENCES

1 Pompeiano M, Tononi G. Changes in pontine muscarinic receptor binding during sleep–waking states in the rat. *Neurosci Lett* 1990; **109** : 347–52.

2 Mash DC, Flynn DD, Kalinoski L, Potter LT. Circadian variations in radioligand binding to muscarine receptors in rat brain dependent upon endogenous agonist occupation. *Brain Res* 1985, **331** : 35–8.

3 Pan SY. Circadian changes of acetylcholine, choline acetyltransferase, acetylcholinesterase and muscarinic receptors in mouse brain. *Acta Pharmacol Sin* 1991; **12** : 148–51

4 Zhang JT, Saito H. Studies on susceptibilities to the

amnestic effects of 12 chemicals on passive avoidance responses in mice: comparison between step down and step through tests. *Acta Pharm Sin* 1986; **21** : 12–9.

5 Pan SY. Influences of intraventricular injection of 6–hydroxydopamine on passive–avoidance response, acetylcholine, and muscarinic receptors in mouse brain. *Acta Pharmacol Sin* 1991; **12** : 344–7.

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

7 Crawley JN. Exploratory behavior models of anxiety in mice. *Neurosci Biobehav Rev* 1985; **9** : 37–44.

8 File SE. What can be learned from the effects of benzodiazepines on exploratory behavior? *Neurosci Biobehav Rev* 1985; **9** : 45–54

9 Piercey MF, Vogelsang GD, Franklin SR, Tang AH. Reversal of scopolamine–induced amnesia and alterations in energy metabolism by the nootropic piracetam: implications regarding identification of brain structures involved in consolidation of memory traces. *Brain Res* 1987; **424** : 1–9.

10 Maquet P, Dive D, Salmon E, Sadzot B, Franco G, Poirrier R, *et al.* Cerebral glucose utilization during sleep–wake cycle in man determined by positron emission tomography and [^{18}F]2–fluoro–2–deoxy–*D*–glucose method. *Brain Res* 1990; **513** : 136–43.

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

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东莨菪碱对小鼠被动学习、探究行为及脑区毒蕈碱受体的昼夜变化

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提要 东莨菪碱(Scop) ip 0.1，0.4 mg · kg^{-1}. 实验于 07:00–09:00，15:00–17:00 和 21:00–23:00 进行. 结果显示，Scop 对小鼠被动学习的抑制作用，及其增加探究活动和减少排便的作用，以白天最明显. 小鼠额叶皮层和海马部位的 M 受体数，白天多于夜晚. 而纹状体 M 受体以上午最少. 结果提示，Scop 对小鼠学习记忆和行为的影响，及其不同脑区的 M 受体，均呈现一定的昼夜变化.

关键词 东莨菪碱；二苯羟乙酸奎宁酯；记忆；探究行为；毒蕈碱受体；额叶；纹状体；海马；昼夜节律

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Specific binding sites of platelet activating factor on the intact bovine cerebral microvascular endothelial cells and antagonism of drugs

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ABSTRACT [^{3}H]Triazolodiazepine ([^{3}H]WEB 2086), an antagonist of platelet activating factor (PAF) receptor, was studied as radioligand on intact cerebral microvascular endothelial cells (CMEC). The results showed that the binding of [^{3}H]triazolodiazepine reached and maintained at an equilibrium after 15–120 min of incubation and that it was saturable with increasing concentration of radioligand. Scatchard analysis indicated that there were 2 specific binding sites on CMEC, its K_{d1}, B_{max1}, K_{d2}, and B_{max2} were 3.13 nmol · L^{-1}, 1.50 pmol / 3 × 10^5 cells, 83.96 nmol · L^{-1}, and 12.96 pmol / 3 × 10^5 cells, respectively. The binding of [^{3}H]triazolodiazepine to CMEC was displaced by C_{16}–PAF and 1,5–bis–(3,4–dimethoxyphenyl)–tetrahydro–(4*H*)–pyran (SZ–1), which IC_{50} were 0.43 nmol · L^{-1} and 0.125 μmol · L^{-1}, respectively. These data suggested the existence of PAF specific binding sites on CMEC.

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