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Effects of suberogorgin and its derivates on learning and memory in mice

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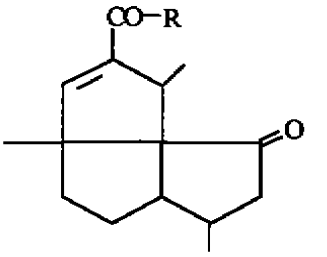
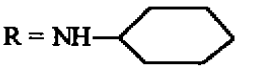
KEY WORDS suberogorgin; *N*-suberogorgamide-*N*-*N*-dicyclohexyl urea; *N*-cyclohexyl suberogorgamide; scopolamine; cycloheximide; learning; memory; cholinesterase inhibitors

AIM: To study the relationship between the effects of suberogorgin (Sub) and its derivates on memory and their anti-acetylcholinesterase (AChE) actions. **METHODS:** The step-down latency (SDL) and the escape latency (EL) of mice were determined at the same time in a passive avoidance task after Sub, *N*-suberogorgamide-*N*-*N*-dicyclohexyl urea (Sub-DU), or *N*-cyclohexyl suberogorgamide (*N*-CS) was injected ip. The AChE activities in brain hemogenates were determined with colorimetry. **RESULTS:** Sub 1.9, Sub-DU 3.0, or physostigmine (Phys) 0.15 mg·kg^{-1} obviously lengthened the SDL by 195 %, 271 %, and 210 %, and shortened the EL by 56 %, 61 %, and 33 %, and the two formers inhibited the brain AChE activities by 17 % and 19 %, respectively in aging (3 - 4 months) mice. These actions were decreased in a dose-dependent manner when Sub or Sub-DU was increased to 2.9 - 4.3 or 4.5 - 6.7 mg·kg^{-1} respectively. Sub 1.9, Sub-DU 2.0, and Phys 0.15 mg·kg^{-1} also lengthened the SDL by 187 %, 209 %, and 152 %, and shortened the EL by 52 %, 62 %, and 57 %, respectively in aged (12 - 14 months) mice. Sub 1.3 - 1.9, Sub-DU 0.9 - 2.0, or Phys 0.15 mg · kg^{-1} reversed the cycloheximide- or scopolamine-induced disruptions of memory retention. No obvious effect of *N*-CS on the acquisition of memory and the AChE activity in mice was observed. **CONCLUSION:** The improvements of Sub and Sub-DU on memory were chiefly related to their anti-AChE actions.

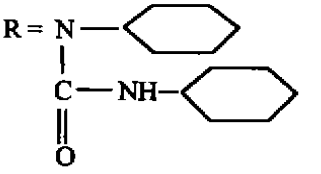
Suberogorgin (Sub) was isolated from *Gorgoniae suberogorgia* sp from South China Sea[1]. Its inhibition on acetylcholinesterase (AChE) was not very strong[2]. *N*-Suberogorgamide-*N*-*N*-dicyclohexyl urea (Sub-DU) and *N*-cyclohexyl suberogorgamide (*N*-CS) were synthesized. The LD_{50} of Sub, Sub-DU, and *N*-CS iv were 23.6, 35.8, and 84.0 mg · kg^{-1}, respectively. The anti-AChE action of Sub-DU was stronger than that of Sub, but that of *N*-CS was very weak. Since some AChE inhibitors can treat the senile dementia[3], the effects of Sub, Sub-DU, and *N*-CS on the memory in normal mice and experimental memory-disrupted mice were studied.



R = OH Sub



N— CS



Sub—DU

MATERIALS AND METHODS

Sub, spectrum pure, was isolated by Department of Chemistry, Sun Yat-Sen University; Sub-DU and *N*-CS, spectrum pure, were synthesized by our laboratory; Physostigmine salicylate (Phys) (Research grade, Serva); Scopolamine hydrobromide (Scop) (Injectio, Guangzhou Qiao Guang Pharmaceutical Factory, China); Cycloheximide (Cyc) (BR, Sigma); Acetylthiocholine iodide (ATCh) (>99 % pure, Fluka); 5, 5′-dithio 2, 2′-dinitrobenzoic acid

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(DTNB) (BR, Shanghai Institute of Biochemistry, Chinese Academy of Sciences); Sodium dodecyl sulphate (SDS) (>99 % pure, BDH).

Aging (3 - 4 months) NIH mice (n = 15 - 28) weighing 22.8 ± s 1.8 g of either sex and aged (12 - 14 months) mice (n = 23) weighing 40 ± s 5 g of ♂ were bred in our laboratory.

Passive avoidance task A wooden box (30 cm × 30 cm × 20 cm) which was separated into 4 equal chambers by black plastic planks. Each chamber had a platform in the right corner. Copper nets (30 V) were laid on the floor. Passive avoidance task was tested[3]. For disruption of memory retention, mice were injected sc with Cyc 150.0 mg·kg^{-1} or Scop 2.0 mg·kg^{-1}, and then ip with test drugs or saline after training. Retention tests were performad 24 or 4 h after Cyc or Scop respectively. The step-down latency (SDL) and the escape latency (EL) of mice were determined. The data were analyzed by ANOVA after 2 longest and 2 shortest latencies in each group were eliminated.

Determination of AChE activity In the experiments of acquisition of memory, 10 mice of each group were randomly sampled, and injected ip with Sub, Sub-DU, N-CS, or saline. After 30 min, their brains were homogenized at 0 ℃. The AChE activity in hemogenates was determined colorimetrically[2].

RESULTS

Memory Thirty min after aging mice were injected ip with Sub 0.6 - 2.9, Sub-DU 0.9 - 4.5, or Phys 0.10 - 0.15 mg · kg^{-1}, their SDL were obviously lengthened and EL were shortened (Tab 1). No obvious effect of N-CS 2.0 - 6.8 mg·kg^{-1} on SDL and EL was observed. In aged mice, Sub 1.3 - 1.9, Sub-DU 1.3 - 2.0, or Phys 0.10 - 0.15 mg · kg^{-1} lengthened the SDL and shortened the EL (Tab 1).

Reversal of Cyc-induced disruption of memory retention Cyc shortened the SDL and lengthened the EL (P<0.01). Cyc + Sub 0.9 - 1.9, Cyc + Sub-DU 0.9 - 2.0, and Cyc + Phys 0.15 mg · kg^{-1} lengthened the SDL and shortened the EL, compared with Cyc + NS; Cyc + Sub 1.9, Cyc + Sub-DU 1.3 - 2.0 mg·kg^{-1} still lengthened the SDL and shortened the EL, compared with saline (Tab 2).

Reversal of Scop-induced transient disruption of short-term memory retention Scop shortened the SDL (P<0.01) and lengthened the EL (P<0.05). Scop + Sub 1.3 - 1.9, Scop + Sub-DU 1.3 - 2.0, and Scop + Phys 0.15 mg·kg^{-1} lengthened the SDL and shortened the EL, compared with Scop + NS; Scop + Sub-DU 1.3 - 2.0, Scop + Phys 0.15 mg · kg^{-1} also lengthened the SDL and shortened the EL, compared with saline (Tab 2).

Tab 1. Effects of suberogorgin (Sub), N-suberogorgamide-N-N-dicyclohexyl urea (Sub-DU), N-cyclohexyl suberogorgamide (N-CS), and physostigmine (Phys) on acquisition of memory of aging (3 - 4 months, n = 28) and aged (12 - 14 months, n = 23) mice in a passive avoidance task before and 30 min after medication.
$\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 mg·kg^{-1}.

	mg·kg^{-1}	Step-down latency/s Before	Step-down latency/s After	Escape latency/s Before	Escape latency/s After
In aging mice					
	0	13 ± 6	21 ± 10	26 ± 12	18 ± 9
Sub	0.6	15 ± 5^a	32 ± 16^b	22 ± 13^a	13 ± 6^b
	0.9	14 ± 5^a	44 ± 26^c	23 ± 10^a	10 ± 6^c
	1.3	12 ± 4^a	59 ± 33^c	25 ± 15^a	8 ± 4^c
	1.9	12 ± 6^a	62 ± 41^c	24 ± 14^a	8 ± 5^c
	2.9	17 ± 9^a	54 ± 42^c	28 ± 15^a	9 ± 7^c
	4.3	15 ± 8^a	22 ± 15^a	28 ± 18^a	16 ± 11^a
Sub-DU	0.9	15 ± 6^a	33 ± 12^b	25 ± 13^a	11 ± 6^c
	1.3	13 ± 3^a	57 ± 25^c	26 ± 15^a	10 ± 4^c
	2.0	13 ± 6^a	65 ± 44^c	26 ± 12^a	8 ± 5^c
	3.0	16 ± 7^a	78 ± 56^c	29 ± 19^a	7 ± 3^c
	4.5	14 ± 6^a	42 ± 24^c	27 ± 16^a	12 ± 9^b
	6.7	14 ± 7^a	27 ± 18^a	22 ± 11^a	21 ± 15^a
N-CS	2.0	13 ± 4^a	19 ± 7^a	26 ± 11^a	16 ± 7^a
	3.0	15 ± 8^a	24 ± 13^a	23 ± 13^a	18 ± 10^a
	4.5	15 ± 6^a	25 ± 11^a	20 ± 12^a	15 ± 11^a
	6.8	16 ± 6^a	20 ± 10^a	27 ± 14^a	20 ± 12^a
Phys	0.10	13 ± 7^a	49 ± 30^c	30 ± 20^a	17 ± 7^a
	0.15	14 ± 3^a	65 ± 46^c	28 ± 10^a	12 ± 6^b
	0.22	15 ± 7^a	41 ± 34^c	24 ± 16^a	13 ± 8^b
In aged mice					
	0	10 ± 6	23 ± 16	28 ± 17	21 ± 11
Sub	1.3	12 ± 8^a	50 ± 29^c	29 ± 14^a	13 ± 6^c
	1.9	10 ± 5^a	66 ± 38^c	25 ± 12^a	10 ± 6^c
Sub-DU	1.3	11 ± 8^a	57 ± 32^c	26 ± 13^a	11 ± 8^c
	2.0	11 ± 7^a	71 ± 48^c	30 ± 14^a	8 ± 4^c
Phys	0.10	10 ± 4^a	52 ± 29^c	27 ± 18^a	15 ± 8^b
	0.15	12 ± 6^a	58 ± 38^c	28 ± 15^a	9 ± 3^c

Inhibition on AChE of brain hemogenates Compared with saline group, Sub 0.9, 1.9, and 4.3 mg · kg^{-1} decreased the AChE activities to 90 ± 9 % (P<0.05), 83 ± 7 % (P<0.01), and 70 ± 9 % (P<0.01), respectively; Sub-DU 1.3, 3.0, and 6.7 mg·kg^{-1} inhibited the AChE activities to 88 ± 6 % (P<0.05), 81 ± 10 % (P<0.01), and 73 ± 8 % (P<0.01), respectively. No inhibition of N-CS 2.0, 4.5, and 6.8 mg·kg^{-1} on

Tab 2. Reversal of cycloheximide (Cyc)- or scopolamine (Scop)-induced disruption of memory retention of aging mice (n = 25 or 15 respectively) in a passive avoidance task. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* NS + NS; $^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* Cyc + NS or Scop + NS.

	mg·kg^{-1}	Step-down latency/s	Escape latency/s
NS + NS		30 ± 19	10 ± 4
Cyc + NS		11 ± 7^c	17 ± 10^c
Cyc + Sub	150.0 + 0.9	25 ± 16^{cf}	10 ± 6^{af}
	150.0 + 1.3	41 ± 18^{bf}	9 ± 6^{af}
	150.0 + 1.9	50 ± 29^{af}	7 ± 5^{af}
Cyc + Sub-DU	150.0 + 0.9	33 ± 21^{af}	8 ± 5^{bf}
	150.0 + 1.3	54 ± 40^{af}	8 ± 3^{bf}
	150.0 + 2.0	60 ± 38^{af}	6 ± 5^{af}
Cyc + Phys	150.00 + 0.10	35 ± 16^{af}	15 ± 10^{ad}
	150.00 + 0.15	49 ± 22^{af}	10 ± 6^{ae}
NS + NS		38 ± 23	12 ± 7
Scop + NS		12 ± 19^c	18 ± 16^b
Scop + Sub	2.0 + 0.9	18 ± 19^{cd}	15 ± 8^{ad}
	2.0 + 1.3	28 ± 19^{af}	10 ± 11^{ae}
	2.0 + 1.9	26 ± 23^{af}	10 ± 9^{ae}
Scop + Sub-DU	2.0 + 0.9	22 ± 18^{be}	10 ± 14^{ad}
	2.0 + 1.3	34 ± 29^{af}	8 ± 8^{bf}
	2.0 + 2.0	39 ± 43^{af}	8 ± 5^{cf}
Scop + Phys	2.00 + 0.10	20 ± 22^{bd}	12 ± 11^{ad}
	2.00 + 0.15	31 ± 17^{af}	9 ± 4^{af}

AChE was seen.

DISCUSSION

The methods of determining the SDL and EL at the same time can eliminate the interferences induced by the unspecific effects of test drugs[3]. The reversals of Sub and Sub-DU on Scop-induced disruption of memory retention indicated that Sub and Sub-DU might act on the memory process specifically.

The memory improvements of Sub and Sub-DU were reduced when they were increased to 2.9 – 4.3 and 4.5 – 6.7 mg·kg^{-1} respectively. These were related to the blockade of nerve induced by too much ACh[3]. *N*-CS, which inhibition on AChE was not obvious, had no effect on memory. The above observations, together with the anti-AChE actions of Sub and Sub-DU, showed that the improvements of Sub and Sub-DU on memory were chiefly related to their inhibitions on AChE. In addition, the results that Sub and Sub-DU reversed the Cyc-induced disruption of memory retention indicated that the anti-AChE action was not the sole mechanism of their memory-improvements.

Irregulation of central cholinergic function induce a disturbance of learning and memory in aged mammals[4]. Alzheimer's disease is related to the defect of cholinergic nerve conduction[3]. The memory-improvements of Sub and Sub-DU in aged mice suggested that both might be investigated as anti-senile-dementia drugs.

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柳珊瑚酸及其衍生物对小鼠学习和记忆的影响

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关键词 柳珊瑚酸；*N*-柳珊瑚酰胺-*N*-*N*-二环己基脲；*N*-环己基柳珊瑚酰胺；东莨菪碱；环己酰亚胺；学习；记忆；胆碱酯酶抑制剂

目的：研究柳珊瑚酸(Sub)、*N*-柳珊瑚酰胺-*N*-*N*-二环己基脲(Sub-DU)，*N*-环己基柳珊瑚酰胺(*N*-CS)在影响记忆与抗胆碱酯酶(AChE)作用间的关系. **方法**：在被动回避操作装置上测定小鼠ip Sub或其衍生物后的跳台潜伏期(SDL)和逃避潜伏期(EL)；比色法测定AChE活力. **结果**：Sub 1.9，Sub-DU 3.0和Phys 0.15 mg·kg^{-1}分别使成年小鼠的SDL延长195 %，271 %和210 %，EL缩短56 %，61 %和33%；前二者尚同时使小鼠全脑匀浆AChE活力各降低17 %和19 % 大于上述剂量时，Sub和Sub-DU对SDL和EL的作用依剂量地减弱. Sub 1.9，Sub-DU 2.0和Phys

0.15 mg·kg^{-1}还可使老年小鼠的 SDL 各延长 187 %，209 %和 152 %，EL 各缩短 52 %，62 %和 57 %. 三药尚明显改善由环己酰亚胺或东莨菪碱产生的记忆保持损害. N-CS 无上述作用. 结论：Sub 和 Sub-DU 对记忆的改善作用与其抗 AChE 有关.

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Effects of calcitonin injected into various brain areas on pain threshold and Ca^{2+} in rats

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KEY WORDS pain threshold; calcitonin; cerebral ventricles; periaqueductal gray; calcium

AIM: To study the effects of calcitonin (Cal) injected into different brain areas on pain threshold. **METHODS**: The analgesic effects of Cal were investigated in rats by the tail-flick test. **RESULTS**: Cal injected into lateral cerebral ventricle (LCV) or periaqueductal gray (PAG) increased obviously the pain threshold to 49 ± 22 % or to 68 ± 12 % ($P < 0.01$), respectively. When PAG was blocked with lidocaine, the analgesic effect of Cal injected into LCV was lowered 41 ± 9 %. Cal injected into habenula (Hab) decreased the pain threshold to − 30 ± 5 % ($P < 0.01$). **CONCLUSION**: Cal in different rat brain areas induced different effects on pain responses: analgesia or hyperalgesia, and showed that PAG played an important role in the analgesic effect induced by Cal, and the changing of pain threshold was mediated by the Ca^{2+} in brain.

Calcitonin (Cal) showed an analgesic action which is involved in the binding of opioid receptors in brain[1]. Periaqueductal gray (PAG) and Habenula (Hab) are the key relays in the realizing of the analgesic action of morphine and acupuncture[2,3]. The Ca^{2+} in CNS antagonizes the analgesic actions of both morphine and acupuncture[4,5]. What is about the role of Cal and what is the relationship between Cal analgesia and Ca^{2+}? To assay this problem will benifit the comprehension of the mechanism of Cal analgesia.

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METHODS

Wistar rats of either sex ($n = 76$) weighing 220 ± 30 g were anesthetized with chloral hydrate 0.4 g·kg^{-1}. Stainless steel guide cannula (OD 0.7 mm, ID 0.4 mm) were inserted into unilateral ventricle, bilateral of PAG, and Hab according to the atlas of Konig & Klippel and the cannula were anchored with dental cement to the skull. A stainless steel cannula (OD 0.4 mm) was in the guide cannula for injection of drugs. After 5 d, experiments were carried out in a quiet room at 20.0 ± 0.5 ℃. The pain threshold were measured by the tail-flick test with a radiant heat sustained for 4 - 5 s. The basal pain threshold was the average of 3 trials before the drug was used.

Cal was injected into LCV 1 μL·min^{-1}, 5 μL; bilateral PAG 1 μL·min^{-1}, 2 μL/side; bilateral Hb 0.25 μL·min^{-1}, 0.5 μL/side.

Injection sites were marked by injecting 2 % pontamine sky blue. The brains were stored in 10 % formalin for at least 5 d. Cannula tip placements were verified from 40 μm coronal sections. Rats were included if the target areas were correct.

The Cal was from Tou You Corporation, Japan, 10 kU·L^{-1} injection. Other drugs were dissolved in saline.

RESULTS

1 Analgesic effect of Cal injected into LCV, PAG and Hab Five minutes after Cal injected into LCV 12.5 - 50 μU in 5 μL the pain threshold was raised, reached the maximum at 20 min, and recovered to the basal level after 1 h. The analgesic