transmission of spinal visceral nociception in the cat. Neurosci Lett 1994; Suppl 45: 23.
9 Bullitt E. Expression of c-*fos*-like protein as a marker for neuronal activity following noxious stimulation in the rat. J Comp Neurol 1990; 296: 517 – 30.
10 Presley RW, Menetry D, Levine JD, Basbaum AI. Systemic morphine suppresses noxious stimulus-evoked Fos protein-like immunoreactivity in the rat spinal cord. J Neurosci 1990; 10: 323 – 35.
11 Leah JD, Sandkuhler J, Herdegen T, Murashov A, Zimmermann M. Potentiated expression of Fos protein in the rat spinal cord following bilateral noxious cutaneous stimulation. Neurosci 1992; 48: 525 – 32.
12 Williams S, Evan GI, Hunt SP. Changing patterns of c-*fos* induction in spinal neurons following thermal cutaneous stimulation in the rat. Neurosci 1990; 36: 73 – 81.
13 Willis WD, Coggeshall RE. Sensory mechanisms of the spinal cord. 2nd ed. New York: Plenum; 1991. p 575 – 9.
14 Sandkuhler J, Treier AC, Liu XG, Ohnimus M. The massive expression of c-*fos* protein in spinal dorsal horn neurons is not followed by long-term changes in spinal nociception. Neuroscience 1996; 73: 657 – 66.

NMDA 受体介导皮下注射福尔马林诱发的大鼠脊髓 Fos 蛋白的表达[1]

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关键词 *N*-甲基-*D*-天冬氨酸受体; 痛觉; 原癌基因蛋白 c-*fos*; 脊髓; 甲醛; 免疫组织化学

目的: 研究 NMDA 和非 NMDA 受体在疼痛刺激诱发脊髓 Fos 表达中的作用. **方法**: 大鼠单侧后足跖部皮下注射 2 % 福尔马林, 免疫组化显示 Fos 的表达. **结果**: 注射福尔马林 2 h 后, Fos 阳性神经元集中分布在同侧脊髓背角 Ⅰ 层的内侧和 Ⅱ 层的浅部. 脊髓鞘内给予 NMDA 受体拮抗剂 APV (10 μL, 0.01, 0.1, 1 g·L^{-1})剂量依赖性引起福尔马林诱发的背角 Fos 阳性细胞数量减少($P < 0.01$); 非 NMDA 受体拮抗剂 DNQX 对 Fos 表达无明显影响. **结论**: NMDA 受体介导福尔马林致痛诱导的脊髓 Fos 表达.

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Effects of aluminum potassium sulfate on learning, memory, and cholinergic system in mice[1]

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KEY WORDS aluminum; alum compounds; AF64A; aziridines; mustard compounds; memory; avoidance learning; acetylcholine; choline acetyltransferase

AIM: To study the relationship between aluminum potassium sulfate (APS) and memory deficits of mice. **METHODS**: 30, 60, or 90 d after the mice were given daily APS ig, the step-through latency (STL) was determined with a passive avoidance task. Aluminum (Al) contents in brain and blood were assayed with atomic absorption spectrophotometry. Acetylcholine (ACh) content in brain was determined with chemiluminescent method and choline acetyltransferase (ChAT) activity was measured radiochemically. **RESULTS**: APS 1 g·kg^{-1} increased blood-Al only after 30 d. After 60 d, STL, ACh content and ChAT activity decreased by 46.4 %, 8.5 %, and 22.6 %, respectively. These parameters decreased by 50 %, 11.1 %, and 27.8 %, respectively, with increased Al in blood and brain, after 90 d. APS 0.25 g·kg^{-1} had no effects on mice except blood-Al. In ethylcholine mustard aziridium chloride (AF64A) treated mice, APS 1 g·kg^{-1}

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only increased blood and brain-Al. **CONCLUSION**: The intake of APS 1 $g \cdot kg^{-1} \cdot d^{-1}$ for 60 d induced learning and memory deficits in mice.

Although the cause of Alzheimer's disease (AD) remains unknown, there is a lot of evidence implicating aluminum as a neurotoxin[1]. Aluminum has been found in increased concentrations in all AD-affected tissues[2]. The toxicity of aluminum depends on its chemical state, the route of administration, and the species of animals[3]. In traditional Chinese medicine, alum [> 99 % of its essential components is $KAl(SO_4)_2 \cdot 12H_2O$, aluminum potassium sulfate (APS)] and some kinds of herbs processed with alum contain aluminum. In this study, APS was tested for its neurotoxicity. AF64A, ethylcholine mustard aziridium chloride, was used for disruption of memory retention in this study. This compound was selected because of its close chemical structural similarity to choline and the presence in the molecule of a cytotoxic aziridium moiety. It is a presynaptic chemical neurotoxin, capable of inducing a persistent deficiency in central cholinergic transmission.

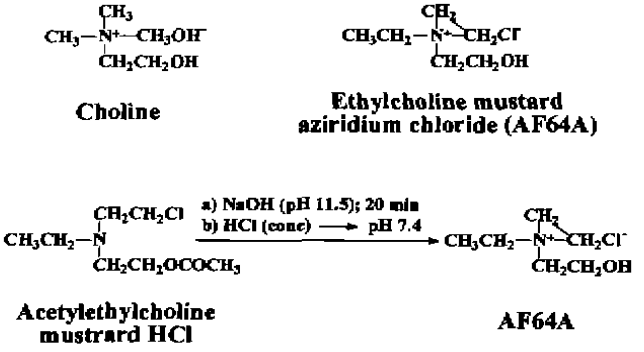

Fig 1. Synthetic pathways used to generate AF64A.

MATERIALS AND METHODS

APS (Beijing Chemical Factory); acetylethylcholine mustard HCl, (acetyl-AF64, chemically pure, was synthesized by Dr LIAO Hong-Biao and Dr LUO Xuan-De, National Institutes of Pharmaceutical Research and Development); luminol (Sigma); horseradish peroxidase (5000 U, Sigma); acetylcholinesterase (1000 U, Fluka); choline oxidase (100 U, Boehringer Mannheim); Triton X-100 (Special pure, Carl Roth KG Chemische Fabrik Karlsruhe); acetyl coenzym A, sodium salt (Sigma); acetyl choline chloride (Sigma); heptanone (GC, Sigma); sodium tetraphenylboron (Sigma); PPO (Sigma); [^{3}H]-acetylCoA (labelled by Prof WU De-Zhu, China Institute of Atomic Energy, Beijing).

NIH mice ♂ ($n = 240$) weighing 22.5 g ± s 1.5 g were obtained from the Experimental Animal Center of the Academy of Traditional Chinese Medicine. (Grade Ⅱ, Certificate № 01-1073).

Passive avoidance task A plastic box was divided into 2 chambers: dark and light. Copper grids (30 V) were put on the floor. The latency of mice staying in light box (STL) was recordered[4]. APS (in tap water) was given ig at 0.25 or 1 $g \cdot kg^{-1} \cdot d^{-1}$ for 30, 60, and 90 d. Water was given to control mice. Solution of AF64A should be prepared with acetylethylcholine mustard HCl (acetyl-AF64) immediately prior to surgery. Acetyl-AF64 is a white, hygroscopic solid. An aqueous solution of acetyl-AF64 was treated with NaOH (10 $mol \cdot L^{-1}$) at pH 11.5 for 20 min to convert the ester to AF64A. The pH was adjusted to 7.4 with concentrated HCl (Fig 1)[5]. Under light anesthesia with ether, AF64A 65 nmol (25 μL per mice) was injected into right ventricle to impair the memory. AF64A was injected icv to 3 groups of mice, in which 1 group was control and the other 2 groups were given APS for 30 d.

Determination of Al The Al content in blood and brain were determined by atomic absorption spectropbotometry[6,7].

Determination of acetylcholine (ACh) and choline acetyltransferase (ChAT) The ACh content in brain was determined with chemiluminescent method. Tissue 20 to 100 mg was extracted in 0.5 to 1 mL of trichloroacetic acid (TCA). The TCA was removed by four or more ether washings until a pH of 4 was reached. The sample (100 μL) was treated with 10 μL of 0.5 % potassium iodate. Dose-response curve solution was prepared with acetylcholine chloride in the range of 10 to 100 $nmol \cdot L^{-1}$ [8].

ChAT activity was assayed with radiochemical method. Homogenates of brain were prepared in 20 times of triton X-100. Thirty min later, it was homogenized again with 2

times of 0.2 mol·L^{-1} phosphate buffer, pH 7.4. The supernatant after centrifugation at 8000 × g for 10 min was used to determine ChAT activity with radiochemical method[9].

As soon as the STL of mice were determined, the mice were decapitated and the brains were frozen at −70 ℃ until the Al, ACh, and ChAT assays.

RESULTS

Memory Not at the end of 30 d, but at the end of 60 and 90 d after the mice were given ig APS 1 g·kg^{-1}, their STL were shortened by 46.4 % and 50 %, respectively, while no obvious effects of APS 0.25 g·kg^{-1} on STL (Tab 1).

Aluminum in blood and brain The blood and brain Al contents increased along with the increase of dose of APS and the time of administration ($P < 0.01$). The Al contents of mice which received APS 1 g·kg^{-1}·d^{-1} for 90 d were the highest, whereas no change of blood and brain Al contents was found in the mice given APS 0.25 g·kg^{-1}·d^{-1} (Tab 1).

ACh content APS 0.25 g·kg^{-1}·d^{-1} exerted no influence on ACh content, while 1 g·kg^{-1}·d^{-1} decreased ACh by 8.5 % and 11.1 % after 60 and 90 d, respectively (Tab 1).

ChAT activity APS 0.25 g·kg^{-1}·d^{-1} showed no obvious effect on ChAT activity of mice, but 1 g·kg^{-1}·d^{-1} of APS decreased ChAT activity by 22.6 % and 27.8 % after 60 and 90 d, respectively (Tab 1).

Effect on AF64A-mice Injecting icv AF64A induced memory disruption of mice along with the changes of STL, ACh, and ChAT (Tab 4). APS did not aggravate memory deficits of AF64A-mice after 30 d. Only APS 1 g·kg^{-1}·d^{-1} increased blood and brain Al ($P < 0.01$), but no obvious effects on memory, ACh content, and ChAT activity were seen (Tab 2).

DISCUSSION

Alum, whose essential component is KAl$(SO_4)_2 \cdot 12H_2O$, is a traditional Chinese medicine

Tab 1. Effects of APS on Al, ACh, ChAT, and STL of mice. $n = 30$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

APS/g·kg^{-1}	STL/s	Blood-Al/mg·L^{-1}	Brain-Al/μg·g^{-1}	ACh/nmol·g^{-1}	ChAT/kBq·g^{-1}·min^{-1}
1st month					
0	246 ± 101	0.09 ± 0.03	1.10 ± 0.49	14.2 ± 1.6	421 ± 41
0.25	260 ± 75	0.17 ± 0.04	1.73 ± 0.16	13.2 ± 0.8	331 ± 43
1	210 ± 105	0.35 ± 0.09	2.54 ± 0.28	13.6 ± 1.4	371 ± 64
2nd month					
0	248 ± 100	0.08 ± 0.03	1.62 ± 0.34	14.2 ± 1.2	434 ± 52
0.25	212 ± 113	0.28 ± 0.07	1.97 ± 0.40	14.0 ± 1.9	391 ± 57
1	133 ± 134^c	0.41 ± 0.08^c	2.80 ± 0.14^c	13.0 ± 0.8^c	336 ± 84^c
3rd month					
0	240 ± 107	0.11 ± 0.04	1.24 ± 0.32	13.5 ± 1.1	392 ± 51
0.25	219 ± 110	0.32 ± 0.07	2.05 ± 0.33	13.9 ± 1.4	382 ± 98
1	120 ± 111^c	0.53 ± 0.09^c	3.52 ± 0.2^c	12.0 ± 1.3^c	283 ± 42^c

Tab 2. Effects of APS on Al, ACh, ChAT, and STL of AF64A-mice after 1-month medication. $n = 30$ mice. $\bar{x} \pm s$. $^cP < 0.01$ *vs* saline icv. $^fP < 0.01$ *vs* AF64A.

	STL/s	Blood-Al/μg·L^{-1}	Brain-Al/μg·g^{-1}	ACh/nmol·g^{-1}	ChAT/kBq·g^{-1}·min^{-1}
Normal	300 ± 0	0.18 ± 0.07	1.10 ± 0.43	14.2 ± 2.3	384 ± 37
Control icv saline	300 ± 0	0.17 ± 0.02	1.27 ± 0.30	14.2 ± 2.8	339 ± 50
AF64A	130 ± 96^c	0.20 ± 0.02	1.14 ± 0.38	10.2 ± 1.7^c	123 ± 60^c
AF64A + APS 0.25 g·kg^{-1}	196 ± 55	0.26 ± 0.06	1.64 ± 0.21	10.1 ± 2.4^c	145 ± 29^c
AF64A + APS 1 g·kg^{-1}	164 ± 121	0.38 ± 0.07^f	2.69 ± 0.46^f	10.4 ± 2.5^c	136 ± 1.9^c

and it is also a kind of food additive in China. Whether aluminum related to Alzheimer's disease is a problem to be resolved. Make sure the safety of alum is important to us all.

The experimental results showed that high dose (1 $g \cdot kg^{-1} \cdot d^{-1}$) and long-term (at least 60 d) intake of APS could induce the learning and memory deficits in mice. ChAT activity and ACh content of the mice were decreased, which were related closely to learning and memory. It is indicated that the neurotoxic effect of APS was on the cholinergic system. But this dose was 40 times over that of humans'. While no effects was found on the behavior of mice at 0.25 $g \cdot kg^{-1} \cdot d^{-1}$ of APS (10 times as humans'). Whether there were some other biochemical changes of mice administered with APS should be studied.

We also studied the effect of aluminum on AF64A-induced disruption of memory retention animal model. After administration of APS for 30 d, no obvious effects on behavioral deficits and choline marker changes was observed. We should research if the administration of APS for longer time could make the memory retention of AF64A mice more serious. We can know whether AF64A-induced learning and memory deficits lead to increased brain affinity to aluminum. Furthermore, we can discover if the increased concentration of aluminum in AD brain was the result of AD-affection or AD was the result of aluminum accumulation.

REFERENCES

1 Lion A. Aluminum toxicology and the aluminum containing medication. Pharmacol Ther 1985; 29: 255.

2 Krishnan S, McLachlan D, Krishnan B. Aluminum toxicity to brain. Sci Total Environ 1988; 71: 59 – 64.

3 WHO Food Additives. Toxicological evaluation of certain food additives and contaminants. Series 24. London: Cambridge University; 1989. p 125.

4 Chen Qi. Experimental methodology of Traditional Chinese Medicine Pharmacology. Beijing: The People's Medical Publishing House; 1994. p 849.

5 Abraham F, Charles R, Ronald J, Isreal H. Long-term central cholinergic hypofunction induced in mice by ethylcholine aziridium ion (AF64A) *in vivo*. Am Soc Pharmacol Exp Ther 1982; 222: 140 – 5.

6 Samir K, Donald H, Peter R. Absorption and disposition of aluminum in the rat. J Pharm Sci 1986; 75: 586 – 9.

7 Keith B, Merle A. Measurement of aluminum in neuronal tissues using electrothermal atomization atomic absorption spectrophotometry. Anal Chem 1986; 58: 1744 – 8.

8 Maurice I, Bernard L. Application to mammaliansch tissue of the chemiluminescent method for detecting acetylcholine. J Neurochem 1982; 39: 3401 – 3.

9 Bertrand N, Beley P, Beley A. Brain fixation for acetylcholine measurements. J Neurosci Methods 1994; 53: 81 – 5.

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硫酸铝钾对小鼠学习、记忆及胆碱能系统的影响[1]

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关键词 铝；铝化合物；AF64A；氮丙啶；芥子化合物；记忆；回避学习；乙酰胆碱；胆碱乙酰基转移酶

目的：研究硫酸铝钾中的铝对正常及 AF64A 小鼠学习、记忆的影响. **方法**：被动回避反射仪测避暗潜伏期(STL)；原子吸收分光光度法测血、脑铝；化学发光法测脑乙酰胆碱(ACh)；放射化学法测脑胆碱乙酰基转移酶(ChAT). **结果**：1 $g \cdot kg^{-1}$ 组 30 日时，血脑铝升高；60 日伴 STL、ACh、ChAT 分别下降 46.4 %，8.5 %，22.6 %；90 日 STL、ACh、ChAT 分别下降 50.0 %，11.1 % 和 27.8 %. 0.25 $g \cdot kg^{-1}$ 只有血铝升高. 1 $g \cdot kg^{-1}$ 使 AF64A 模型小鼠血、脑铝升高，其他无改变. **结论**：硫酸铝钾 1 $g \cdot kg^{-1} \cdot d^{-1}$ 60 日使小鼠学习、记忆障碍及胆碱能系统改变.