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目的: 观察银杏内酯 A 和 B, PAF 拮抗剂阿帕泛 (Apa) 和 NOS 抑制剂 L-NA 对新生大鼠小胶质细胞 (Mi) 产生 NO 的影响. 方法: 以 Griess 反应测定亚硝酸盐含量表示 NO 量. 结果: 在静息 Mi, GA, GB 和 Apa 在 $1-10\,000\text{ nmol}\cdot\text{L}^{-1}$ 范围对 Mi 产生 NO 没有影响, 但 L-NA 可浓度依赖性地抑制 NO 产生, 其 IC_{50} (95% 可信限) 值为 $3.4 (0.8-14.9)\ \mu\text{mol}\cdot\text{L}^{-1}$. 而在激活的 Mi, GA, GB 和 L-NA 可浓度依赖性地抑制 NO 产生, 其 IC_{50} (95% 可信限) 值分别为 $5.7 (1.8-18.1)$, $1.1 (0.3-4.4)$ 和 $0.5 (0.1-2.8)\ \mu\text{mol}\cdot\text{L}^{-1}$, 但 Apa 不能抑制 NO 产生. 结论: GA 和 GB 抑制 LPS 诱导 Mi 产生 NO.

467-470 银杏内酯抑制新生大鼠小胶质细胞产生一氧化氮

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关键词 银杏内酯; 血小板激活因子; 硝基精氨酸; 一氧化氮; 小胶质细胞; 脂多糖; 培养的细胞; 阿帕泛

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Nitric oxide and soman poisoning

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KEY WORDS soman; acetylcholine; nitric oxide; arginine; nitric-oxide synthase; *N*^G-nitro-*L*-arginine methyl ester

AIM: To examine whether nitric-oxide (NO) is involved in the toxicity of soman. **METHODS:** With pretreatments of icv *L*-arginine (Arg, the substrate of nitric-oxide synthase NOS), *N*^G-nitro-*L*-arginine methyl ester (NAME, the inhibitor of NOS), the latency of seizure, and the mortality of mice induced by soman poisoning were examined. The activities of brain NOS in soman-intoxicated mice were measured. **RESULTS:** In case of Arg pretreatments, the latency decreased ($P < 0.05$) from (5.2 ± 1.8) min (control) to (4.3 ± 0.8) min (Arg 160 nmol), and the mortality increased ($P < 0.05$) from 50% (control) to 81% (Arg 160 nmol). In case of NAME pretreatment, the latency increased ($P < 0.01$) from (4.0 ± 1.1) min (control) to (14.5 ± 5.0) min (NAME 2.20 μmol), and the mortality decreased ($P < 0.05$)

from 87% (control) to 50% (NAME 2.20 μmol). The toxicity of soman in mice was enhanced by Arg and reduced by NAME all in a dose-dependent fashion. NAME antagonized the enhancement of soman poisoning by Arg. Intoxication of mice with soman increased the NOS activity in cerebrum, cerebellum, and hippocampus from 100% to 104% ($P < 0.05$), 115% ($P < 0.01$), and 111% ($P < 0.01$), respectively. **CONCLUSION:** The onset of seizure and death of mice induced by soman poisoning are related to the NO messenger system.

Soman is a potent inhibitor of acetylcholinesterase. The enzyme loses its activity after being phosphorylated, hence the released acetylcholine accumulates at the synaptic cleft, consecutively stimulates the cholinergic receptors causing so-called hypercholinergy.

Being a highly reactive free radical, nitric-oxide (NO) manifests cytotoxic action in neurons intoxicated by many compounds^[1-3]. We are intriguing in whether NO is also involved in soman intoxication.

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MATERIALS AND METHODS

Materials Kunming mice (18 - 22 g, medical animal No 01-3023, Laboratorial Animal Center, Academy of Military Medical Sciences) were used. *N*^G-nitro-*L*-arginine methyl ester (NAME), soybean trypsin inhibitor, leupeptin, and aprotinin were purchased from Sigma. Phenylmethylsulfonyl fluoride (PMSF) and HEPES were purchased from Serva. Soman purity was >98%. Other reagents were CP.

Soman toxicity The skull of mouse was exposed by making a T-shape incision at the scalf. A needle was inserted into the right cerebroventricle in a depth of 2.0 mm in front of the lambdoid suture and 1.5 mm aside the sagittal suture. Aliquots (5 μ L) of *L*-arginine (Arg) and NAME dissolved in Alder's solution (NaCl 100, KCl 5.4, CaCl₂ 3.6, MgCl₂ 2.6, NaH₂PO₄ 1.0, NaHCO₃ 41.8, glucose 7.6 mmol \cdot L⁻¹, pH 7.4) were injected icv before sc injection of soman. To the intoxication control mice aliquots (5 μ L) of Alder's solution were injected icv. Latency between sc soman and the onset of seizure as well as the mortality in 24 h were recorded.

Determination of NOS activity Mice injected sc with soman 0.14 mg \cdot kg⁻¹ were killed when seizure appeared. The cerebrum, cerebellum, and hippocampus were cooled on ice and homogenized with HEPES buffer 10 mmol \cdot L⁻¹ containing sucrose 0.32 mol \cdot L⁻¹, edetic acid 0.1 mmol \cdot L⁻¹, dithiothreitol 1 mmol \cdot L⁻¹, soybean trypsin inhibitor 10 mg \cdot L⁻¹, leupeptin 10 mg \cdot L⁻¹, aprotinin 2 mg \cdot L⁻¹, and PMSF 1 g \cdot L⁻¹, pH 7.4 (1:5 wt/vol for cerebrum; 1:10 wt/vol for cerebellum and hippocampus). The homogenates were spun at 10 000 \times g for 3 min. The supernatants were stored on ice and the NOS activity was determined^[4] immediately.

RESULTS

Potential of soman toxicity by Arg

In the control group 15/30 mice died with vigorous convulsion after soman intoxication (0.15 mg \cdot kg⁻¹, sc) with a latency of (5.2 \pm 1.8) min. The latency to onset in mice receiving Arg 160 nmol before intoxication was shortened, and the mortality obviously increased along with the increasing dosages of Arg (Tab

1). Mice injected Arg or citrulline alone up to 160 nmol did not show any symptom within 6 h examined.

Tab 1. Effect of pretreatment of arginine on soman poisoning in mice. Arg 5 μ L icv, 5 min prior to soman 0.15 mg \cdot kg⁻¹ sc intoxication. $\bar{x} \pm s$. ^a*P* > 0.05, ^b*P* < 0.05 vs vehicle, Dunnett *t* test. ^d*P* > 0.05, ^e*P* < 0.05 vs vehicle, χ^2 test.

Arg/ nmol	Latency to onset/ min	Mortality Death/Total	%
0	5.2 \pm 1.8	15/30	50
20	5.7 \pm 1.4 ^a	8/18 ^d	44
40	5.3 \pm 1.7 ^a	11/19 ^d	58
80	4.2 \pm 1.0 ^a	19/23 ^e	83
160	4.3 \pm 0.8 ^b	21/26 ^e	81

Protection of soman intoxication by

NAME Aliquots (5 μ L) of various concentrations of NAME in Alder's solution were injected icv in mice. NAME 0.55 and 1.10 μ mol did not cause any change in the mouse behavior, whereas 1.65 and 2.20 μ mol induced a slightly sedative effect in mice. In the control group, same volumes of Alder's solution without NAME were injected icv to the mice, 20/23 mice died of soman challenging (0.18 mg \cdot kg⁻¹, sc) 10 min later with convulsion. Mice pretreated with NAME survived against soman intoxication in a dose-dependent fashion, and the latency to onset was prolonged (Tab 2).

Tab 2. Effect of pretreatment of NAME on soman poisoning in mice. NAME 5 μ L icv, 10 min prior to soman 0.18 mg \cdot kg⁻¹ sc intoxication. $\bar{x} \pm s$. ^a*P* > 0.05, ^b*P* < 0.05, ^c*P* < 0.01 vs vehicle, Dunnett *t* test. ^d*P* > 0.05, ^e*P* < 0.05 vs vehicle, χ^2 test.

NAME/ μ mol	Latency to onset/ min	Mortality Death/Total	%
0	4.0 \pm 1.1	20/23	87
0.55	5.9 \pm 1.4 ^a	11/15 ^d	73
1.10	8.1 \pm 1.8 ^b	8/15 ^e	53
1.65	8.8 \pm 2.9 ^c	8/15 ^e	53
2.20	14.5 \pm 5.0 ^c	9/18 ^e	50

NAME antagonism of Arg effect on soman poisoning In intoxication group, 12/25 mice receiving Alder's solution without Arg or NAME died of poisoning. Mortalities of mice of the Arg controls (80 and 160 nmol, 5 min prior

to soman intoxication) raised up to 67 % and 83 %, respectively, while mice of the NAME control (1100 nmol, 10 min prior to soman intoxication) declined to 20 % as expected. In microinjections with NAME and Arg into the same ventricle in tandem 10 min and 5 min before soman challenging, NAME 1.1 μmol completely antagonized the effect of Arg 80 nmol, or partially blocked that of 160 nmol (Tab 3).

Tab 3. Antagonism of the effect of Arg by NAME on soman poisoning in mice. Arg and NAME (5 μL of each, icv) were microinjected 5 and 10 min before soman poisoning (sc 0.14 mg · kg⁻¹) respectively. $\bar{x} \pm s$. * $P < 0.01$ vs soman control, t test. ^a $P < 0.05$ vs soman control, χ^2 test.

	Dosage/ nmol	Latency to onset/min	Mortality Death/Total	%
Soman control	0	4.7 ± 1.5	12/25	48
Arg control	80	4.9 ± 1.1	10/15	67
	160	4.1 ± 0.8	15/18 ^a	83
NAME control	1 100	10.3 ± 3.2 ^a	6/30 ^a	20
Arg + NAME	80 + 1 100	7.0 ± 1.6 ^a	7/15	47
	160 + 1 100	8.2 ± 2.4 ^a	9/15	60

Brain NOS activities in soman poisoned mice Mice intoxicated with soman (0.14 mg · kg⁻¹, sc) were killed as soon as the seizure appeared. Intoxication of mice with soman increased the NOS activity in cerebrum, cerebellum, and hippocampus from 100 % to 104 % ($P < 0.05$), 115 % ($P < 0.01$), and 111 % ($P < 0.01$), respectively. The NOS activity of cerebrum, cerebellum, and hippocampus in control mice were (1.20 ± 0.15), (2.86 ± 0.26), and (1.68 ± 0.28) nmol · g⁻¹, respectively.

DISCUSSION

We have determined that acetylcholine significantly elevated the free intrasynaptomal Ca²⁺, and the effect could be blocked by atropine (result not shown). It is known that free Ca²⁺ rising up to concentrations higher than 400 nmol · L⁻¹ would activate the NOS which keeps inactivated at the resting state^[5], and NOS could catalyze Arg oxidation producing nitric oxide^[6], an important signaling molecule in a variety of physiological and pathological events. In the present experiments, we demonstrated that

the toxicity of soman in mice could be increased by pretreatment with the NO donor Arg, and reduced by NAME, the inhibitor of NOS. It implies that NO plays an important role in the soman intoxication. The mechanism of the action of NO deduced from the above-mentioned data seems to be as follows: soman — AChE ↓ — ACh ↑ — M-AChR — Ca²⁺ ↑ — NOS ↑ — NO ↑ — soman toxicity ↑.

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一氧化氮与索曼中毒

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关键词 索曼; 乙酰胆碱; 一氧化氮; 精氨酸; 一氧化氮合酶; 硝基-精氨酸甲酯

目的: 探查索曼中毒机制是否有 NO 参与. **方法:** 预先侧脑室注射 Arg、NAME, 观察索曼中毒小鼠惊厥潜伏期、死亡率及脑中 NOS 活性变化. **结果:** Arg 预处理时, 潜伏期从 5.2 min (对照组) 缩短到 4.3 min (Arg 160 nmol), 死亡率由 50 % (对照组) 增加至 81 % (Arg 160 nmol). NAME 预处理时, 潜伏期从 4.0 min 延长到 14.5 min (NAME 2.20 μmol), 死亡率由 87 % (对照组) 减少至 50 % (NAME 2.20 μmol). 索曼的小鼠毒性被 Arg 增强、被 NAME 减弱, 均有剂量依赖性. 索曼中毒可使小鼠大脑、小脑及海马的 NOS 活性分别增加 4 %, 15 % 及 11 %. **结论:** 索曼中毒小鼠的惊厥及死亡与 NO 信使系统有关.

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