Effect of sodium dimercaptopropanesulfonate on antagonism of tetramethylenedisulphotetramine to GABA receptor

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KEY WORDS sodium dimercaptopropanesulfonate; tetramethylenedisulphotetramine; GABA receptors; autoradiography

ABSTRACT

AIM: To study effects of sodium dimercaptopropanesulfonate (DMPS) on the antagonism of tetramethylenedisulphotetramine (TETS) to γ-aminobutyric acid (GABA) receptor. METHODS: Acute toxicity experiments were conducted to observe the effects of DMPS and TETS on mice. Contents of free amino acids in mouse brain were determined with automatic analyzer for amino acids. Autoradiography was used to observe the [3H]GABA binding in the rat brain slices under different conditions. RESULTS: After ivc and ip DMPS, the number of mice experiencing convulsions reduced from 20 in control group to 4 and 2 respectively in TETS poisoned mice. The content of GABA was altered in DMPS control group and TETS control group compared with DMPS protection group and NS control group (μmol/g; (2.09 ± 0.05) and (2.67 ± 0.15) vs (2.40 ± 0.10 (μmol/g)) and (2.41 ± 0.21)); the content of glutamic acid was (12.3 ± 1.2), (12.0 ± 0.8), (10.2 ± 0.6), and (11.8 ± 1.0) μmol/g in NS control group, DMPS control group, TETS control group, and DMPS protection group, respectively. The OD value of autoradiograms decreased in TETS group compared with buffer control group in cortex, hippocampus, diencephalon, and brainstem [(0.084 ± 0.008), (0.081 ± 0.009), (0.094 ± 0.006) and (0.081 ± 0.006), vs (0.102 ± 0.003), (0.109 ± 0.005), (0.128 ± 0.007), and (0.125 ± 0.008), respectively]. OD value was maintained or higher than the normal level in DMPS + TETS group in the four brain areas [(0.116 ± 0.008), (0.125 ± 0.011), (0.129 ± 0.005), and (0.125 ± 0.010) vs (0.102 ± 0.003), (0.109 ± 0.005), (0.128 ± 0.007), and (0.125 ± 0.008), respectively]. CONCLUSION: The inhibitory effects of DMPS on the antagonism of TETS to GABA receptor are due to the increase in the GABA binding to its receptors in brain caused by DMPS.

INTRODUCTION

Tetramethylenedisulphotetramine (TETS), commonly called, Dushuiqiang, Sanbudao, and Meishunqing in Chinese, is a kind of effective neurotoxic rodenticide. As a rodenticide, TETS has been used for more than forty years, but lacks selectivity and has been found to be a hypotoxic compound for all warm-animal species. Specific antitode has not been found, therefore, the production of TETS has been prohibited by our government and others. TETS, however, has the characteristics of involving simple synthetic technology, low price, and good poisoning effects on rats. Recently, TETS has been produced, sold, and used illegally among the people since the plague of rats was serious in parts of our country and the rodenticide market was chaotic. Cases of intoxication and death induced by TETS have been reported repeatedly.1,2 According to incomplete statistics, TETS which accounted for 22 % rodenticide used in rural in 1996, has caused nearly one thousand intoxication and one hundred deaths because of eating by mistake3. TETS has strong excitatory effects on the central nervous system (CNS) and the death results from the respiratory failure induced by tetanic convulsion in poisoned animals.4 Smythies suggested that TETS antagonized γ-aminobutyric acid (GABA)-depolarization and might be a GABA antagonist5. Our latest study demonstrates that sulphydryl compounds such as sodium dimercaptopropanesulfonate (DMPS), sodium dimercapto-succinate, L-cysteine, and dithiothreitol have an antitodal action on

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acutely TETS poisoned animals up to varying degrees\(^{6}\). Recent reports have shown that exogenous sulfhydryl compounds may modulate the functional characteristics of various ionicotropic receptors and ion channels\(^{7}\). The anticonvulsant mechanisms of sulfhydryl compounds on non-metallic compounds through influencing GABA receptor have not been reported so far. Therefore, we chose the most effective compound, DMPS, to study further.

MATERIALS AND METHODS

Drugs TETS, Sanbuda, was extracted and purified by chemical methods and identified by the Second Institute of the Ministry of Public Security (purity: 90.5 %). Sodium dimercaptopyrrolenine sulphonate injection was obtained from Shanghai Hefeng Pharmaceutical Co (Batch: 971003). \[^{[4,4^{-3}H]}\text{GABA} (36 \text{nmol/L, 1.04 TBq/mol})\] was purchased from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. Amino acids standard were from Sigma Chemical Co, and Tris was obtained from Boehringer Co. The drugs were made fresh daily using distilled water. Volumes of ip injection was 10 mL/kg.

Animals Kunming (KM) strain mice (weighing 21.1 g ± 0.9 g) and Sprague-Dawley (SD) rats (weighing 227 g ± 13 g) were supplied by the Experimental Animal Centers of both Wenzhou Medical College (Grade II, Certificate No 2209001) and Beijing Medical University (Grade II, Certificate No 01-3055 (SD rats) and 01-3050 (KM mice)).

Acute toxicity experiments Mice were randomly divided into 3 groups, 20 mice/group (10 \(\hat{\Phi}\) and 10 \(\hat{\varphi}\)). The DMPS experimental group was injected i.v. with DMPS 3 mg/kg. Other 2 groups were injected ip NS and ip DMPS 300 mg/kg, respectively. Each group was injected ig with TETS 0.3 mg/kg. Volume of i.v. injections was 0.5 mL/kg (the dose and time of administration were ascertained according to the most suitable conditions through previous experiments). The site of i.v. administration was left 2 mm of sagittal suture on the linking line between ears. The poisoning symptoms were observed and the mortality was recorded within 3 d.

Free amino acid analysis in the mouse brain According to previous experiments, doses of ig TETS and ip DMPS were 0.3 mg/kg and 300 mg/kg. Mice were divided into 4 groups (\(\hat{\Phi}\), 6 mice/group): NS control group (ip NS at 25–30 min before decapitation), TETS control group (the death time was 25–30 min after ig TETS and mice were decapitated immediately on death), DMPS control group (decapitated at 55–60 min after ip DMPS), DMPS experimental group (ip DMPS at 30 min before ig TETS, decapitation at 25–30 min after ig TETS). The mouse head was frozen quickly on dry ice. Their brains were taken out rapidly and homogenized in 1 mL ice-cold 4 % sulfosalicylic acid in a glass homogenizer. The homogenate was centrifuged at 4 °C, 10,000 \(\times\) g for 30 min. The supernatant fluid was collected and stored at 20 °C. The contents of free amino acid were measured by automatic analyzer for amino acids (Hitachi 835-50, Japan).

Preparation of brain slices Rats (6 \(\hat{\varphi}\)) were killed by decapitation, and the whole brains were rapidly dissected and frozen on dry ice. The brains were sectioned coronally (Bregma: -0.4 mm and 4.4 mm, 8 pieces/site) with Reichert histostat (Germany) at 20 °C. Two slices were thaw-mounted onto a gelatin-coated slide, stored at -20 °C for 24 h prior to incubation.

Radioligand binding and autoradiography The binding conditions were as described in the literature\(^{6,9}\) and modified according to the results of trial tests. The endogenous GABA was removed by preincubating the slices in Tris-HCl (pH 7.4) buffer for 50 min at 22 °C. The brain slices were incubated with \([4,4^{-3}H]\) GABA (1.2 nmol/L) for 50 min at 22 °C in the presence of Tris-HCl buffer only, DMPS (300 \(\mu\)mol/L), TETS (200 \(\mu\)mol/L), and DMPS + TETS, respectively. DMPS and TETS were dissolved in Tris-HCl buffer. After the incubation, slices were washed twice for 30 s in buffer and once for 15 s in distilled water at 4 °C. Slices were then dried by airing under a fan at room temperature and exposed with plastic \([\hat{\varphi}]\) to Hyperfilm-\(\hat{\varphi}\) (Amersham) for 5 weeks at 4 °C. The optical densities were measured in different brain areas of autoradiograms using an image processing and analysis system (Leica Q500 FW, Germany). Brain structures were identified referring to the rat atlas\(^{10}\).

Statistical analysis Mean values are given as \(\bar{x} \pm s\). Difference between two groups was detected using t-test or Chi-square test. The data for multiple comparisons were analyzed with the Dunnett’s test.

RESULTS

Antidotal actions of DMPS on acutely TETS poisoned mice In mice ip treated with NS, spasm, holding up of head and tail, bounding backwards, and
then tetanic convulsions were observed within 5 min after Ig TETS. The cause of death was respiratory arrest induced by tetanic seizure (the thoracic cavity was opened up and the heart beat continued for 1 – 2 min after the respiratory arrest). In mice treated with both DMPS 300 mg/kg ip and DMPS 3 mg/kg icv, no apparent effects were seen, and the number of convulsive seizures and mortality reduced markedly (P < 0.01) compared with control group (Tab 1).

**Effect of DMPS and TETS on contents of free amino acids in mouse brain** Content of free GABA increased and free Glu reduced (P < 0.01) in TETS control group, and contents of GABA and Glu showed no significant difference in DMPS protection group, compared with NS control group. DMPS could maintain the normal level of GABA and Glu in TETS poisoned mouse brain (Tab 2).

**Effect of DMPS and TETS on [3H]GABA binding in rat brain areas** The distribution of GABA receptors in rat brain areas had differences under normal conditions. TETS inhibited [3H]GABA binding in cortex, hippocampus, diencephalon, and brainstem. DMPS increased [3H]GABA binding in the first three areas. The [3H]GABA binding maintained the normal level in brainstem and diencephalon and increased in cortex and hippocampus in DMPS + TETS group (Fig 1, 2, Tab 3).

**DISCUSSION**

TETS is a stable compound which detains in the animal organism by the original shape and can cause the second intoxication when eaten by people. Previous experiments have shown that TETS is essentially a central nervous system stimulant with greatest action on the brainstem (4) and causes reduction in the maximum depolarization of GABA in the isolated superior cervical ganglion of

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**Tab 1. Effects of DMPS on convulsion induced by TETS (Ig, 0.3 mg/kg) in mice. n = 20. s x ± s. P < 0.01 vs NS control group.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose/ mg·kg⁻¹</th>
<th>Time/min</th>
<th>Route</th>
<th>Conv. number</th>
<th>Death time/ min</th>
<th>Mort. Death/Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMPS protection</td>
<td>300</td>
<td>30 before Ig TETS</td>
<td>ip</td>
<td>2</td>
<td>168 ± 39</td>
<td>2/20</td>
<td>10</td>
</tr>
<tr>
<td>DMPS protection</td>
<td>3</td>
<td>1 after Ig TETS</td>
<td>icv</td>
<td>4</td>
<td>137 ± 27</td>
<td>4/20</td>
<td>20</td>
</tr>
</tbody>
</table>

**Tab 2. Effects of DMPS (ip, 300 mg/kg) and TETS (Ig, 0.3 mg/kg) on the contents of free amino acid in mouse brain. n = 6. s x ± s. P < 0.05, P < 0.01 vs NS control group. P < 0.05, P < 0.01 vs TETS group.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Gly</th>
<th>GABA</th>
<th>Free amino acid/μmol·g⁻¹</th>
<th>Tau</th>
<th>Glu</th>
<th>Arg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NS control</td>
<td>1.33 ± 0.03</td>
<td>2.41 ± 0.21</td>
<td>15.0 ± 1.2</td>
<td>12.3 ± 1.2</td>
<td>0.148 ± 0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TETS control</td>
<td>1.32 ± 0.14</td>
<td>2.67 ± 0.15</td>
<td>14.6 ± 0.6</td>
<td>10.2 ± 0.6</td>
<td>0.149 ± 0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMPS control</td>
<td>1.22 ± 0.06</td>
<td>2.09 ± 0.05</td>
<td>15.1 ± 1.4</td>
<td>12.0 ± 0.8</td>
<td>0.141 ± 0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMPS protection</td>
<td>1.11 ± 0.09</td>
<td>2.40 ± 0.10</td>
<td>14.8 ± 1.1</td>
<td>11.8 ± 1.0</td>
<td>0.140 ± 0.017</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tab 3. Bindings of [3H]GABA in rat brain areas in four pilot groups. Optical density determination was used to estimate the GABA receptor density. n = 12. s x ± s. P < 0.01 vs buffer control group. P < 0.01 vs TETS control group.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Diencephalon</th>
<th>Brainstem</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer control</td>
<td>0.102 ± 0.003</td>
<td>0.109 ± 0.005</td>
<td>0.123 ± 0.007</td>
<td>0.125 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>DMPS control</td>
<td>0.148 ± 0.021</td>
<td>0.127 ± 0.019</td>
<td>0.153 ± 0.006</td>
<td>0.131 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>TETS control</td>
<td>0.084 ± 0.006</td>
<td>0.081 ± 0.009</td>
<td>0.094 ± 0.006</td>
<td>0.081 ± 0.006</td>
<td></td>
</tr>
<tr>
<td>DMPS + TETS experimental</td>
<td>0.116 ± 0.006</td>
<td>0.123 ± 0.011</td>
<td>0.129 ± 0.005</td>
<td>0.128 ± 0.010</td>
<td></td>
</tr>
</tbody>
</table>
Fig 1. Autoradiograms of [3H]GABA binding on TETS (A, B) and DMPS (C, D) group. A and C: Bregma - 4.4 mm; B and D: Bregma - 0.4 mm.

the rat). So, TETS may be acting as an antagonist to the GABA receptors. GABA is a major inhibitory neurotransmitter in the mammalian central nervous system and mediates approximately 40% of inhibitory neurotransmission. In this study, the results of amino acids analysis showed that TETS increased the content of free GABA and decreased the content of free Glu in brain and the autoradiogram of TETS group illustrated that the most significant inhibition on GABA receptor was in the brainstem. The increase in GABA levels diminished the responsiveness of the GABA receptor to subsequent stimulation by GABA, thereby diminished the chloride flux response. Therefore, the convulsive actions of TETS had something to do with its actions that enhanced the content of free GABA and reduced the binding of GABA to receptor.

Sulphydryl groups play a major role in maintaining normal structure and function of many proteins and enzymes and can maintain the redox state of intracellular thiol groups and the function of thiol proteins. Sulphydryl compounds might modify many proteins and low molecular weight compounds via thiol/disulfide exchange reactions and serve as neuromodulators other than as antioxidants and free radical scavengers. The present experiments indicated that DMPS reduced the content of GABA and elevated the OD value of autoradiogram in DMPS group. Consequently, DMPS, as an exogenous compound containing 2 free sulphydryl groups, could potentiate the binding of GABA receptor.

The anticonvulsant actions of DMPS on TETS might be involved in the influence of GABA receptor since the toxic effects of TETS are mainly in the central nervous system. Sulphydryl compounds could participate in mediation of functions through various mechanisms, such as stabilizing the reactive sulphydryl group on the transport protein oriented toward the external surface of the plasma membrane and regulating the binding sites of receptor through sulphydryl/disulfide groups in protein subunits of GABA receptor. Our results showed that iv traces of DMPS had good anticonvulsive actions which were not significantly different as compared with ip DMPS 300 mg/kg. DMPS maintained the content of free GABA within the normal levels in TETS poisoned mice brains and the binding of GABA receptor with ligand increased.
in brain slices coincubated by DMPS + TETS.

In conclusion, our results demonstrate that the inhibitory effects of DMPS on the antagonism of TETS to GABA receptor are due to the properties of DMPS increasing the binding of GABA to receptor.

REFERENCES


15. Allan AM, Bajer LD. Effect of thiol group modification on ion flux and ligand binding properties of the GABA_A-benzodi-


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关键词 二硫丙磺钠；四次甲基二硫四胺；GABA 受体；放射自显影术

目的：探讨二硫丙磺钠（DMPS）对四次甲基二硫四胺（TETS）拮抗 GABA 受体的影响。方法：通过急性毒性试验观察 DMPS 和 TETS 对小鼠的影响；利用氨苯丁酸自动分析仪测定小鼠脑内游离氨基酸的变化；通过宏图放射自显影术观察不同实验组中 [3H]-GABA与其受体结合情况。结果：与 NS 对照组比较，ivc 和 ip DMPS 后小鼠的惊厥数从 20 只下降至 4 只和 2 只；小鼠脑内游离 GABA 含量与 DMPS 保护组和生理盐水组比较，DMPS 对照组和 TETS 对照组含量均有变化 [μmol/g；(2.09 ± 0.05) 和 (2.67 ± 0.15)，vs (2.40 ± 0.10) 和 (2.41 ± 0.21)]；在 NS 对照组，DMPS 对照组和 TETS 对照组中，小鼠脑内游离的谷氨酸 (Glu) 含量分别是 (12.3 ± 1.2)，(12.0 ± 0.8)，(10.2 ± 0.6) 和 (11.8 ± 1.0) μmol/g。与缓冲液对照组大鼠脑片中不同脑区 [(0.102 ± 0.003)，(0.109 ± 0.005)，(0.128 ± 0.007) 和 (0.125 ± 0.008)] 之间，TETS 组放射自显影影像中大鼠皮层、海马、间脑、脑干的光密度显著降低 [(0.084 ± 0.008)，(0.081 ± 0.009)，(0.094 ± 0.006)，和 (0.081 ± 0.006)]；DMPS + TETS 组中不同脑区的光密度 [(0.116 ± 0.008)，(0.125 ± 0.011)，(0.129 ± 0.006) 和 (0.128 ± 0.010)] 维持或高于正常水平 [(0.102 ± 0.003)，(0.109 ± 0.005)，(0.128 ± 0.007) 和 (0.125 ± 0.008)]。结论：DMPS 通过增加脑内 GABA 与受体的结合发挥抑制 TETS 拮抗 GABA 受体的作用。

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