Inhibitory effects of succinic acid on chemical kindling and amygdala electrical kindling in rats¹

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ABSTRACT

AIM: To investigate the effects and mechanism of succinic acid on pentylenetetrazol (PTZ) chemical kindling and amygdala electrical kindling in rats. METHODS: PTZ chemical kindling and amygdala electrical kindling models were established in rats. The effects of succinic acid on the behavior and afterdischarge of kindled rats were observed. The mice were pretreated with succinic acid, 30 min later, picrotoxin, a GABA_A receptor antagonist was given by ip, then the effects of succinic acid on mice were observed. RESULTS: Succinic acid (100-400 mg/kg, ip) dose-dependently inhibited PTZ chemical and amygdala kindled seizure (P<0.05, P<0.01), elevated the afterdischarge threshold, and reduced the Racine’s stage of amygdala kindling rats (P<0.05, P<0.01); succinic acid (200-400 mg/kg, ip) inhibited picrotoxin-convulsion in mice (P<0.05, P<0.01). CONCLUSION: Succinic acid inhibits PTZ chemical and amygdala electrical kindling in rats, and the inhibition mechanism may be related to the enhancement of GABAergic system action in the brain, especially through GABA_A receptors.

INTRODUCTION

Succinic acid was so named because it is found mainly in amber, a famous mineral pharmacy¹¹, which is also found in organisms such as aquatic alga. It has been demonstrated that there is a specific glucose metabolic by-pass in the brain, glutamic acid-GABA-succinic acid by-pass. Its inhibitory effects on central nervous system and anti-convulsion property have been reported²¹. However, antiepileptic effect of succinic acid has not been reported. The present study was designed to investigate the antiepileptic effects of succinic acid and mechanism on PTZ chemical and amygdala electrical kindling in rats.

MATERIALS AND METHODS

Drugs Succinic acid was produced by Beijing Chemical Plant(China); pentylenetetrazol (PTZ), picrotoxin, and pentobarbital were purchased from Sigma (St Louis, USA).

Animals Wistar rats (♀, weighing 180 ±10 g)
were provided by the Animal Center, Qingdao Institute of Drug Control (Certificate No. 000697, Grade II). Four rats per cage were under controlled temperature (23 ± 2°C) and on a 12 h-light/12 h-dark lighting cycle with free access to food and water. Kunming mice (♀, weighing 20 ± 2 g) were from Animal Center, Qingdao Institute of Drug Control (Certificate No. 000209, Grade II).

**Chemical kindling** PTZ (1.75% solution) were administered to rats, 35 mg/kg (subconvulsive dosage) ip, and the behavioral seizure severity was recorded within 1 h after administration. Seizures induced were classified into 7 behavioral categories (grade 0-6) according to Ono [4]: grade 0, no behavioral seizures; grade 1, head nodding or twitching; grade 2, myoclonic jerks; grade 3, head twisting, forelimb clonic convulsions; grade 4, kangaroo position; grade 5, falling down; grade 6, tonic convulsions. Rats which displayed 5 consecutive grade 2 or higher seizures were defined as kindled rats.

The kindled rats (n = 9) were given succinic acid of various doses (50, 100, 200, and 400 mg/kg, ip) at a volume of 2 mL/kg. After 30 min of injection, the afterdischarge threshold (ADT) was determined with the ramp method. A constant-voltage stimulus (1 V) was delivered to determine ADT. And then the voltage was increased by 0.2 V every 2 min till an afterdischarge of at least 3 s duration was evoked. Afterdischarge, seizure percentage, and seizure severity were monitored at the ADT. The rats were given the same volume of NS as self-control before they were given succinic acid. The interval of experiments was at least 4 d.

**Effect of succinic acid on mouse picrotoxin-convulsion** Kunming mice (n = 30) were randomly divided into three groups (n = 10). Succinic acid-treated groups were treated with succinic acid (200 and 400 mg/kg), while the control group were given the same volume of NS. Then 30 min later, the mice were administered picrotoxin, a GABA$_A$ receptor antagonist, 7.5 mg/kg ip [9]. Picrotoxin was diluted in Me$_2$SO.

**Statistical analysis** Data were expressed as mean±SD. Intergroup differences were analyzed using unpaired and paired t-test.

**RESULTS**

**Inhibition of succinic acid on PTZ chemical kindling** Succinic acid (100-400 mg/kg, ip) decreased Ono grade 6 percentage (P < 0.05, P < 0.01) and dose-dependently inhibited PTZ chemical kindling seizure (P < 0.05, P < 0.01). The dose of 50 mg/kg ip, had no effect on kindling seizure compared with the controls (Tab 1).

**Effect of succinic acid on behavioral seizures in amygdala kindled rats** Succinic acid (50 mg/kg, ip) had no effect on kindled rats. The dose of 100-400
mg/kg ip increased ADT ($P<0.05, P<0.01$), reduced Racine stage ($P<0.05, P<0.01$), and dose-dependently decreased seizure severity ($P<0.05, P<0.01$) (Tab 2).

Tab 2. Effects of succinic acid on ADT and behavior seizure in amygdala kindled rats. $n=9$ rats. Mean±SD. $^aP>0.05, ^bP<0.05, ^cP<0.01$ vs individual vehicle control.

<table>
<thead>
<tr>
<th>Dose/</th>
<th>ADT/V</th>
<th>Racine stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg·kg$^{-1}$</td>
<td>Vehicle</td>
<td>Succinic acid-treated</td>
</tr>
<tr>
<td>50</td>
<td>3.4±1.0</td>
<td>3.4±1.1$^a$</td>
</tr>
<tr>
<td>100</td>
<td>3.4±1.2</td>
<td>3.8±1.0$^b$</td>
</tr>
<tr>
<td>200</td>
<td>3.4±1.1</td>
<td>3.8±1.0$^b$</td>
</tr>
<tr>
<td>400</td>
<td>3.4±1.0</td>
<td>3.9±1.1$^c$</td>
</tr>
</tbody>
</table>

Effect of succinic acid on picrotoxin induced convulsion succinic acid (200 and 400 mg/kg, ip) prolonged the latency of picrotoxin-induced convulsion in mice ($P<0.05, P<0.01$, Tab 3).

Tab 3. Effect of succinic acid on mice picrotoxin-convulsion. $n=10$ mice. Mean±SD. $^bP<0.05, ^cP<0.01$ vs individual vehicle control.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose/</th>
<th>Convulsant</th>
<th>Latency/</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg·kg$^{-1}$</td>
<td>animals</td>
<td>min</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>10</td>
<td>9±3</td>
<td></td>
</tr>
<tr>
<td>Succinic acid-treated</td>
<td>200</td>
<td>9</td>
<td>14±5$^b$</td>
</tr>
<tr>
<td>400</td>
<td>8</td>
<td>17±3$^c$</td>
<td></td>
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</table>

rats is considered to be a good model of human chronic epilepsy.

The present study showed that succinic acid (100-400 mg/kg, ip) dose-dependently inhibited PTZ chemical kindling and amygdala electrical kindling, decreasing Ono seizure severity and the percentage of Ono grade 6 in PTZ chemical kindling; elevating the ADT and reducing seizure severity and Racine stage in amygdala electrical kindling. Succinic acid (200-400 mg/kg, ip) prolonged the latency of convulsant mice caused by picrotoxin, which suggests succinic acid has inhibitory effect on GABA$_A$ receptor, according with the enhancement effect on GABAergic system of semicarbazide convulsant model reported in the preamble.

Succinic acid is similar in molecular structure to inhibitory amino acids neurotransmitter GABA and excitatory amino acid neurotransmitter glutamic acid. It is interesting that in structure it is also similar to sodium oxybate, a clinical local anesthetic. Excitatory and inhibitory neurotransmitters, such as glutamic acid and GABA, take part in maintaining the balance of excitation and inhibition in the brain, where there is a specific by-pass of succinic acid. This suggests that succinic acid is an endogenous neuro-active substance. Succinic acid may act through GABA receptor or through glutamic acid receptor. To investigate the inhibitory effect of succinic acid on kindling, we studied its effect on picrotoxin-induced convulsion according to Zhang et al. The results indicated that the inhibitory effect of succinic acid on kindling probably involved the enhancement of GABA$_A$ subtype receptor, while the inhibitory effect on glutamic acid receptor could not be
excluded.

In conclusion, succinic acid inhibits the PTZ chemical kindling and amygdala electrical kindling, and the mechanism is related to the enhancement of the GABAergic system action in the brain. Since succinic acid has low toxicity, it is of pharmacological value to further study the anti-epileptic effect of succinic acid.

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REFERENCES