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Inhibitory effects of succinic acid on chemical kindling and amygdala electrical kindling in rats[1]

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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:** Succinc 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 pharmacy[1], 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 reported[2]. 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 g±10 g)

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

Chemical kindling PTZ (1.75 % solution) were administered to rats, 35 mg/kg[3] (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. Succinic acid was diluted in normal saline (NS). The day before that, the rats were given equal volume of NS as self-control. Behavioral seizure severity was recorded within 1 h and seizure percentage on grade 6 was calculated.

Amygdala kindling The rats were anesthetized with sodium pentobarbital (40 mg/kg, ip) and given stereotaxic implantation of a bipolar electrode into each basolateral nucleus of the amygdala. The electrode consisted of two twisted stainless-steel wires (0.25 mm in diameter) which were separated at the tip by 0.25 mm. According to the brain atlas of König *et al*[5], the following stereotaxic coordinates were used: AP 3.0 mm, L 4.8 mm, DV 8.8 mm. All coordinates were measured from bregma. The electrode pair was anchored to the skull with miniature screws and dental cement. Following the electrode implantation, the animals were treated with sodium benzylpenicillin for 3 d to prevent infection.

Two weeks after the operation, constant current stimulations (400 μA, 60 Hz, 1 ms, monophasic square wave for 1 s) were delivered to the right amygdala once a day. EEG and seizure stage were recorded and analyzed. Behavioral seizure severity was classified according to the classification described by Racine[6]: stage 1, facial clonus; stage 2, head nodding; stage 3, bilateral forelimb clonus; stage 4, rearing; stage 5, rearing and falling. Animals which displayed 5 consecutive seizures on stage 5 were defined as kindled.

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 monitered 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[7] 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[8], a $GABA_A$ receptor antagonist, 7.5 mg/kg ip[9] . Picrotoxin was diluted in Me_2SO.

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

Tab 1. Effect of succinic acid on behavioral seizure in PTZ kindled rats. *n*=9 rats. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* individual vehicle control.

Dose/ mg·kg^{-1}	Ono grade		Ono grade 6 seizure percentage/%	
	Vehicle	Succinic acid-treated	Vehicle	Succinic acid- treated
50	6.0	5.3±1.0[a]	100.0	66.7[a]
100	6.0	4.6±1.1[b]	100.0	25.0[b]
200	6.0	3.3±1.4[c]	100.0	16.7[c]
400	6.0	3.0±1.3[c]	100.0	12.5[c]

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. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* individual vehicle control.

Dose/ mg·kg^{-1}	ADT/V		Racine stage	
	Vehicle	Succinic acid-treated	Vehicle	Succinic acid- treated
50	3.4±1.0	3.4±1.1[a]	5.0	4.2±1.6[a]
100	3.4±1.2	3.8±1.0[b]	5.0	3.1±1.5[b]
200	3.4±1.1	3.8±1.0[c]	5.0	1.9±1.2[c]
400	3.4±1.0	3.9±1.1[c]	5.0	1.5±0.8[c]

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. [b]*P*<0.05, [c]*P*<0.01*vs* individual vehicle control.

Group	Dose/ mg·kg^{-1}	Convulsant animals	Latancy/ min
NS		10	9±3
Succinic acid-treated	200	9	14±5[b]
	400	8	17±3[c]

DISCUSSION

PTZ chemical kindling and amygdala electrical kindling models are two commonly used kindling models. The former is an ideal model in the study of the grand mal of human primary epilepsy[10], while the latter is similar to human epilepsy in seizure activity, EEG, and the epileptiform discharge[11]. The kindling model in 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[1].

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[12]. 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*[7] . 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[13] , it is of pharmacological value to further study the anti-epileptic effect of succinic acid.

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琥珀酸对大鼠化学性点燃和杏仁核电刺激性点燃的抑制作用[1]

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关键词 点燃效应; 癫痫; 琥珀酸; 印防己毒素; GABA-A 受体

目的: 研究琥珀酸对大鼠戊四唑化学性点燃(kindling)发作及杏仁核电刺激点燃发作的影响及作用机制. 方法: 建立大鼠戊四唑化学性点燃模型和杏仁核电刺激点燃癫痫模型, 测定琥珀酸对点燃发作的脑电活动及行为变化指标的影响. 测定琥珀酸对$GABA_A$受体拮抗剂印防己毒素诱发小鼠惊厥的影响. 结果: 琥珀酸(100-400 mg/kg, ip)对两种点燃模型有显著抑制作用, 降低发作强度和全身性发作百分率($P<0.05$, $P<0.01$), 可升高杏仁核电刺激点燃大鼠的局灶性后放电阈值($P<0.05$, $P<0.01$), 以上反应呈剂量效应关系。琥珀酸可延长印防己毒素诱发小鼠惊厥的潜伏期($P<0.05$, $P<0.01$). 结论: 琥珀酸对大鼠戊四唑化学性点燃和脑杏仁核电刺激点燃发作有抑制作用, 其机制可能与增强$GABA_A$受体功能有关.

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