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Inhibition of nicardipine on amygdala kindling in rats[1]

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KEY WORDS nicardipine; calcium channel blockers; amygdaloid body; kindling (neurology); electric stimulation; GABA; epilepsy

ABSTRACT

AIM: To investigate the effects and mechanism of nicardipine, a calcium channel blocker, on amygdala kindling in rats. **METHODS**: Constant current stimulations were delivered to the right amygdala of the rats once a day. The effects of nicardipine on the development and seizure of amygdala kindling were observed and the content of amino acid neurotransmitters in the brain of kindled rats was determined. **RESULTS**: Nicardipine (2 mg·kg^{-1}, ip) significantly retarded the development of amygdala kindling ($P < 0.01$); 2 - 20 mg·kg^{-1} of nicardipine inhibited amygdala kindled seizure dose-dependently, elevated the afterdischarge threshold, and reduced the Racine's stage; 20 mg·kg^{-1} of nicardipine significantly increased the content of GABA, a kind of inhibitory amino acid neurotransmitter, in the brain of amygdala kindled rats ($P < 0.05$). **CONCLUSION**: Nicardipine inhibits amygdala kindling in rats, and the inhibition mechanism may be related to the blockage of the voltage-operated calcium channels and the enhancement of GABAergic system action in the brain.

INTRODUCTION

It has been demonstrated that the influx of calcium, which probably depends upon the activation of voltage-operated calcium channels[1], is involved in the genesis of neuronal epileptogenic activity, and several studies have examined the effects of calcium channel antagonists on some animal models of epilepsy[2,3]. Nicardipine, a dihydropyride calcium channel blocker, is used mainly to treat cardiovascular and cerebrovascular diseases. Antiepileptic effect of nicardipine has not been reported. Most antiepileptic drugs, such as phenobarbiturates and phenytoin, can block calcium influx of the neurons and enhance GABAergic system action[4]. The present study was designed to investigate the effects and the mechanism of nicardipine on the development and seizure of amygdala kindling in rats.

MATERIALS AND METHODS

Drugs Nicardipine and sodium pentobarbital were purchased from Sigma (St Louis, USA). Dimethylsulphoxide (Me_2SO) was produced by Shanghai Hengda Chemical Co (China). Standard mixed amino acid was obtained from Hitachi Co (Osaka, Japan).

Animals Female Wistar rats weighing (180 ± 10) g 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.

Implantation of electrodes The rats were anesthetized with sodium pentobarbital (40 mg·kg^{-1}, ip) and given stereotaxic implantation of one 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 Konig *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. After electrode implantation, the animals were treated with sodium benzylpenicillin for 3 d to prevent animals from infection.

Amygdala kindling Two weeks after the operation, constant current stimulations (400 μA, 60 Hz, 1 ms, monophasic square wave for 1 s) were dilivered to the right amygdala once a day. Afterdischarge duration

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(ADD) was 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 had displayed 3 consecutive Stage 5 seizures were defined as kindled.

Effect of nicardipine on the development of amygdala kindling Nicardipine was diluted with Me_2SO. The rats were randomly divided into two groups ($n = 10$). One group was given nicardipine (2 $mg \cdot kg^{-1}$, ip), and the other group, the control group, was given the same volume of Me_2SO 30 min prior to kindling stimulations. The rats were treated for 7 consecutive days. The stimulations were continued until all the rats developed Stage 5 seizure.

Effect of nicardipine on behavioral seizure in kindled rats The kindled rats ($n = 10$) were given nicardipine of various doses (2, 5, 7.5, 10, and 20 $mg \cdot kg^{-1}$, ip) at a volume of 2 $mL \cdot kg^{-1}$. After 30 min of injection, afterdischarge threshold (ADT) was determined by the ramp method. A constant-voltage stimulus (1 V) was delivered and then repeated at a 3-min interval increasing each time in a 0.2 V step till an afterdischarge of at least 3 s duration was evoked[7]. Afterdischarge, ADD and seizure severity were monitered at the ADT. The rats were given the same volume of Me_2SO as self-control before they were given nicardipine. The interval of experiments was at least 4 d.

Effect of nicardipine on the content of amino acid neurotransmitters in the brain of amygdala kindled rats The healthy Wistar rats were normal control group ($n = 6$). Kindled rats were divided into a kindled group ($n = 6$) and a kindled nicardipine-treated group ($n = 6$). The control group and model group were treated with Me_2SO (2 $mL \cdot kg^{-1}$, ip). The nicardipine-treated group was treated with nicardipine (20 $mg \cdot kg^{-1}$, ip). The rats were killed 30 min after drug treatment. The brains were removed, wrapped with aluminium foil, and preserved at −85 ℃ till amino acid determination.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed by t-test.

RESULTS

Inhibition of nicardipine on amygdala kindling development Electric stimulations (20 ± 4) numbers were required to elicit Stage 5 seizure in the presence of nicardipine (2 $mg \cdot kg^{-1}$, ip), while (13 ± 5) numbers of electric stimulations were required in the control group ($P < 0.01$). Nicardipine-treated animals required more stimulations to progress through each stage of behavioral seizures. The ADD was the same in nicardipine and vehicle treated animals (Tab 1).

Tab 1. Effect of nicardipine (2 $mg \cdot kg^{-1}$, ip) on characteristics of kindling development in rats. $n = 10$ rats. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* vehicle control.

Stage	M_2SO-treated		Nicardipine-treated	
	Stimulation numbers	ADD/s	Stimulation numbers	ADD/s
1	2 ± 1	8 ± 4	5 ± 2[b]	9.6 ± 2.9[a]
2	7 ± 2	18 ± 9	10 ± 3[c]	19 ± 9[a]
3	8 ± 3	29 ± 12	15 ± 3[c]	34 ± 6[a]
4	11 ± 5	38 ± 14	18 ± 3[c]	45 ± 13[a]
5	13 ± 5	59 ± 24	20 ± 4[c]	59 ± 21[a]

Effect of nicardipine on behavioral seizures in kindled rats Nicardipine (2, 5, 7.5, 10, and 20 $mg \cdot kg^{-1}$, ip) significantly inhibited amygdala kindled seizure, increased ADT, and reduced seizure severity dose-dependently ($P < 0.01$, Tab 2).

Tab 2. Effect of nicardipine (2.0 – 20.0 $mg \cdot kg^{-1}$, ip) on behavioral seizure in kindled rats. $n = 10$ rats. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* individual vehicle control.

Dose/ $mg \cdot kg^{-1}$	ADT/V		Racine's stage	
	Vehicle	Nicardipine-treated	Vehicle	Nicardipine-treated
2.0	3.3 ± 1.1	3.4 ± 1.1[a]	5.0	4.2 ± 1.6[a]
5.0	3.5 ± 1.1	3.9 ± 1.0[b]	5.0	1.9 ± 1.5[c]
7.5	3.5 ± 1.1	3.9 ± 1.1[b]	5.0	1.0 ± 1.1[c]
10.0	3.5 ± 1.1	4.0 ± 1.1[c]	5.0	1.0 ± 0.8[c]
20.0	3.4 ± 1.1	4.2 ± 1.3[c]	5.0	0.6 ± 0.7[c]

Nicardipine had a stable time-course effect after 30 min of administration, significantly elevated ADT, and decreased seizure severity ($P < 0.05$). The maximal effect time was 30 min.

Effect of nicardipine on the content of amino acid neurotransmitters in the brain of amygdala kindled rats Nicardipine (20 $mg \cdot kg^{-1}$, ip) significantly increased content of GABA in the brain of kindled

rats ($P < 0.05$), while there was no difference between the normal control and model group. Nicardipine (20 mg·kg^{-1}, ip) had no effect on inhibitory neurotransmitters (glycine and taurine) or on excitatory glutamic and aspartic neurotransmitters (Tab 3).

DISCUSSION

Kindling refers to a phenomenon in which repeated and periodic administration of initially subconvulsive electric stimulations results in progressive intensification of seizure activity and culminates in a generalized clonic motor seizure. The kindling model in rats is considered to be a reasonably good model of complex partial epilepsy in human[8].

The present study showed that nicardipine (2 mg·kg^{-1}) significantly retarded the development of kindling and indicated an inhibitory effect on the propagation of kindled epileptogenic activity. Nicardipine (2 – 20 mg·kg^{-1}) dose-dependently inhibited amygdala kindled seizures, elevated the afterdischarge threshold, and reduced the seizure severity, which suggests nicardipine have antiepileptic effects. Entry calcium is considered to be involved in the genesis of epileptiform activity[9] and the entry of calcium is probably mediated through the operation of voltage-operated calcium channels[1]. The dilatory effect of nicardipine on the blood vessels of brain would enhance its antiepileptic action[10]. Kindling leads to the enhancement of voltage-dependent Ca^{2+} uptake, in addition to the decrease in GABAergic neurotransmission[11]. It demonstrated that nicardipine could inhibit the chemical kindling induced by pentylenetetrazole (in press). We determined the effect of nicardipine on the content of amino acid neurotransmitters in the brain of amygdala kindled rats. The result showed that nicardipine (20 mg·kg^{-1}) increased the content of inhibitory amino acid neurotransmitter, GABA, but had no effect on other inhibitory and excitatory amino acid neurotransmitters in the brain of kindled rats.

In conclusion, nicardipine inhibits the amygdala kindling, and the inhibition mechanism is related to the blockage of the voltage-operated calcium channels and the enhancement of the GABAergic system action in the brain.

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Tab 3. Effects of nicardipine (20 mg·kg^{-1}, ip) on brain amino acid neurotransmitter content in amygdala kindled rats (μmol·g^{-1} wet tissue). $n = 10$ rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* model control.

Group	GABA	Taurine	Glycine	Glutamine	Aspartate
Normal control	6.2±0.9	3.2±0.6	2.0±0.3	16.2±1.9	4.4±0.3
Kindled group	6.5±0.6	3.2±1.2	2.0±0.2	16.5±3.0	4.5±0.4
Nicardipine-treated	7.6±0.9^b	2.6±0.6	1.9±0.1	16.9±0.9	4.3±0.1

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尼卡地平对大鼠杏仁核点燃效应的抑制作用[1]

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关键词 尼卡地平; 钙通道阻滞药; 杏仁核; 点燃效应(神经病学); 电刺激; GABA; 癫痫

目的: 探讨钙通道阻滞剂尼卡地平对大鼠杏仁核点燃的作用及机制. **方法**: 恒定电流每日一次刺激大鼠右侧杏仁核, 观察尼卡地平对杏仁核点燃发展和发作的影响, 测定点燃大鼠脑内各种氨基酸神经递质的含量. **结果**: 腹腔注射尼卡地平 2 $mg\cdot kg^{-1}$显著延缓杏仁核点燃发展进程($P<0.01$); 尼卡地平 2 $-20\ mg\cdot kg^{-1}$剂量依赖性抑制大鼠杏仁核点燃发作, 升高发作阈值(ADT), 降低 Racine 分级; 20 $mg\cdot kg^{-1}$尼卡地平显著提高杏仁核点燃大鼠脑内抑制性氨基酸神经递质 GABA 的含量($P<0.05$). **结论**: 尼卡地平对大鼠杏仁核点燃的发展和发作有抑制作用, 其机制可能与阻断电压依赖性钙通道及增强 GABA 系统功能有关.

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