Effect of combination of dizocilpine with general antiepileptic drugs on amygdala kindling models in rats

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Abstract: **Aim** To investigate the antiepileptic effect of dizocilpine (MK-801) on amygdala kindling models in rats and the effects of its combination with general antiepileptic drugs . **Methods** To establish amygdala kindling models in rats and observe the effect of dizocilpine on kindling models and its combination with general antiepileptic drugs (phenobarbital , valproate and nicardipine) at ineffective dose . The influence of dizocilpine on convulsions induced by semicarbazide (SCZ) in mice were also observed . **Results** Dizocilpine (0.1 - 0.25 mg•kg^{-1} , ip) was shown to dose-dependently inhibit amygdala kindled seizure , shorten the after discharge duration (ADD) and reduce the Racine's stage ($P < 0.01$) . The combination of dizocilpine with phenobarbital , valproate , nicardipine at ineffective dose shortened ADD or reduced Racine's stages ($P < 0.01$) . Dizocilpine (0.1 - 0.25 mg•kg^{-1} , ip) significantly prolonged the latency and reduced the rate of convulsions and death in mice . **Conclusion** Dizocilpine inhibits the seizure of the amygdala kindling and improve the antiepileptic activity of phenobarbital , valproate and nicardipine , indicating that these combination may provide a new approach for treating epilepsy .

Key words: dizocilpine ; amygdala kindling ; epilepsy ; phenobarbital

CLC number: R917 .6 ; R965 **Document code**: A **Article ID**: 0513 - 4870(2004)02 - 0089 - 04

MK-801 与抗癫痫药合用对大鼠杏仁核点燃的影响

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摘要 : 目的 在大鼠杏仁核点燃模型研究 MK-801 (地佐西平) 及其联合用药的抗癫痫作用 。方法 建立大鼠杏仁核慢性电刺激点燃模型 ,测定不同剂量的 MK-801 对点燃模型各项指标的影响 ,探讨 MK-801 与其他抗癫痫药的协同作用 ,用氨基脲诱发的小鼠惊厥模型测定 MK-801 抗惊厥作用 。结果 MK-801 (0.1 ~ 0.25 mg•kg^{-1}) 可剂量依赖性抑制杏仁核点燃 ,缩短后放电时程 ,降低 Racine's 分级 ;在对点燃均无明显影响的剂量下 , MK-801 (0.05 mg•kg^{-1}) 与抗癫痫药(苯巴比妥 、丙戊酸及尼卡地平) 合用可缩短后放电时程或降低 Racine's 分级 。MK-801 (0.1 ~ 0.25 mg•kg^{-1}) 显著降低小鼠氨基脲诱发的发作潜伏期 、惊厥发生率和死亡率 。结论 MK-801 具有抑制大鼠杏仁核点燃的作用 ,增强苯巴比妥 、丙戊酸及尼卡地平的抗癫痫活性 ,为临床的合并用药提供实验依据 。

关键词 : 地佐西平 ; 杏仁核点燃 ; 癫痫 ; 苯巴比妥

Epilepsy is one of the most common nervous system diseases . However , the mechanism of onset is not clear . Dizocilpine (MK-801) is a non-competitive antagonist of *N*-methyl-*D*-aspartate (NMDA) receptor , which can easily penetrate blood-brain barrier[1] . It has been known that epileptic seizure is related to the balance between excitatory transmitters and inhibitory transmitters[2,3] . In this study the antiepileptic effect of dizocilpine on amygdala kindling in rats , which resembles complex partial epilepsy was investigated[2,4] .

Received date : 2003-03-07 .

Foundation item : Educational Committee of China ([1995]806) .

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Materials and methods

Drugs Dizocilpine , nicardipine , valproate and semicarbazide (SCZ) were purchased from Sigma (St Lous , USA) ; phenobarbital from Mingtai Drug Co.Ltd in Jilin province ; sodium penicillin from Ha' erbin Drug Co. Ltd ; sodium pentobarbital from (Union Co. Ltd) . All drugs were dissolved in saline except nicardipine which was dissolved in Me_2SO.

Animals Wistar rats (♀ , weighing 190 g ±10 g) were supplied by the Animal Center , Qingdao Institute of Drug Control (Certificate No. 000697 , Grade II) . They were housed in a controlled environment (temperature 23 ±2 ℃) on a 12 h light - 12 h dark cycle with free access to food and water. Kunming mice (♂♀ , weighing 20 g ±2 g) were also from the Animal Center , Qingdao Institute of Drug Control (Certificate No. 000209 , Grade II) . Experiment was routinely performed between 14∶00 - 17∶00 in a quiet laboratory .

Epilepsy models The rats were anesthetized with sodium pentobarbital (40 $mg\cdot kg^{-1}$, ip) and a bipolar electrode was stereotaxically implanted into each basolateral nucleus of the amygdala . The electrode was made of two pieces of 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 . After electrode implantation , the animals were treated with sodium penicillin for 3 d to prevent infection[6] .

After a postoperative recovery period of 2 weeks , all rats received the kindling stimulus once daily , which consisted of a 1s train of 60 Hz , 400 μA and monophasic square wave pulses at the intensity described below[7,8] . After discharge duration (ADD) was the total time of spikes in the EEG recorded from the stimulation . The development of kindled seizures was assessed by using a modification of Racine' s stage as follows[9] : stage 0 , no response or behavior arrest ; stage 1 , rhythmic mouth and facial movement ; stage 2 , rhythmic head nodding ; stage 3 , forelimb clonus ; stage 4 , rearing bilateral forelimb clonus ; stage 5 , rearing and falling . Animals which displayed 3 consecutive seizures on stages 5 were defined as kindled .

Inhibitory effect of dizocilpine on previously amygdala kindled rats The kindled rats were given dizocilpine (0.05 , 0.1 and 0.25 $mg\cdot kg^{-1}$, ip) at a volume of 2 $mL\cdot kg^{-1}$. Two hours after administration the ADD and Racine' s stage of the dizocilpine on amygdala kindling models were recorded . The rats were previously given the same volume of saline as control . The interval of experiments was at least 4 d .

Effects of dizocilpine combined with phenobarbital , valproate or nicardipine on amygdala kindled rats Dizocilpine (0.05 $mg\cdot kg^{-1}$, ip) combined with ineffective dose of phenobarbital (7.5 $mg\cdot kg^{-1}$, ip) , valproate (200.0 $mg\cdot kg^{-1}$, *po*)[10] , nicardipine (2.0 $mg\cdot kg^{-1}$, ip) , ADD and Racine' s stage were recorded according to the evaluation criterion . The rats received the same volume of saline as selfcontrol before the administration of the medicine .

Influence of dizocilpine on SCZ-induced seizure Forty mice were divided randomly into 4 groups and were given saline or dizocilpine (0.05 , 0.1 and 0.25 $mg\cdot kg^{-1}$, ip) at a volume of 20 $mL\cdot kg^{-1}$. SCZ (150 $mg\cdot kg^{-1}$, iv) were administrated at a volume of 10 $mL\cdot kg^{-1}$. The latency of seizure and rate of onset and death were recorded 3 h later[11] .

Statistical analysis All data obtained were expressed as $\bar{x}\pm s$. Intergroup differences were analyzed using paired *t*-test . Enumeration data were analyzed by Chi-square test .

Results

1 Inhibitory effect of dizocilpine on amygdala kindled rats

As shown in Table 1 , dizocilpine significantly suppressed both the seizure stage and ADD in a dose-dependent manner .

Table 1 Inhibitory effect of dizocilpine (ip) on amygdala kindled rats

Dose / $mg\cdot kg^{-1}$	ADD / s		Racine' s stage	
	Vehicle	Dizocilpine treated	Vehicle	Dizocilpine treated
0.05	71 ±18	61 ±26	5 ±0	4.6 ±0.6
0.10	90 ±17	71 ±27*	5 ±0	4.3 ±0.7*
0.25	58 ±14	22 ±10**	5 ±0	2.2 ±0.8**

$n=9$, $\bar{x}\pm s$. * $P<0.05$, ** $P<0.01$ *vs* individual vehicle control . ADD: After discharge duration

2 Effects of dizocilpine combined with phenobarbital, valproate or nicardipine on amygdala kindled rats

As shown in Table 2, when ineffective dose of dizocilpine was combined with phenobarbital, valproate, nicardipine on amygdala kindled rats the ADD or Racine's stage were reduced ($P < 0.05$).

Table 2 Effects of dizocilpine (ip) combined with phenobarbital (ip), valproate (*po*), nicardipine (ip) on amygdala kindled rats

Group	Dose / mg·kg^{-1}	ADD / s		Racine's stage	
		Vehicle	Treated	Vehicle	Treated
Dizocilpine	0.05	71 ± 18	61 ± 26	5 ± 0	4.6 ± 0.6
Phenobarbital	7.5	96 ± 10	92 ± 12	5 ± 0	4.7 ± 0.4
	10.0	106 ± 4	68 ± 10*	5 ± 0	4.2 ± 0.1*
Dizocilpine + phenobarbital	0.05 + 7.5	91 ± 18	62 ± 26*	5 ± 0	4.3 ± 0.7*
Valproate	200	90 ± 11	99 ± 25	5 ± 0	4.4 ± 1.1
	500	72 ± 19	34 ± 13*	5 ± 0	2.8 ± 1.8*
Dizocilpine + valproate	0.05 + 200	97 ± 16	87 ± 16*	5 ± 0	4.3 ± 0.8*
Nicardipine	2.0	60 ± 39	63 ± 35	5 ± 0	5 ± 0
	5.0	74 ± 14	69 ± 13	5 ± 0	1.9 ± 1.5*
Dizocilpine + nicardipine	0.05 + 2.0	95 ± 17	46 ± 22**	5 ± 0	4.0 ± 1.3

$n = 9$, $\bar{x} \pm s$. * $P < 0.05$, ** $P < 0.01$ *vs* individual vehicle control

3 Influence of dizocilpine on SCZ-induced convulsion in mice

Convulsion induced by SCZ in mice can be classified into 4 stages: 1, clonic; 2, running; 3, hindlimb tonic; 4, death. Table 3 shows dizocilpine (0.05 - 0.25 mg·kg^{-1}, ip) can significantly prolong the latency and reduce the rate of seizure and death.

Table 3 Influence of dizocilpine (ip) on SCZ-induced convulsion in mice

Group	Dose/ mg·kg^{-1}	Latency/ min	Seizure rate/ %	Death rate/ %
Vehicle		72 ± 9	100	90
Dizocilpine	0.05	76 ± 10	100	80
	0.10	128 ± 30**	80	50*
	0.25	169 ± 20**	30**	30**

$n = 10$, $\bar{x} \pm s$. * $P < 0.05$, ** $P < 0.01$ *vs* individual vehicle control. SCZ: Semicarbazide

Discussion

It is known that excitatory amino acid neurotransmitters (such as glutamate and aspartate) and inhibitory amino acid neurotransmitters should be kept in balance, otherwise, epilepsy may result[2]. Dizocilpine can dose-dependently inhibit amygdala kindled seizure, shorten ADD and reduce the Racine's stage of amygdala kindling rats. Dizocilpine is a non-competitive NMDA (exicitatory amino acid neurotransmitter) receptor antagonist. Dizocilpine is suggested to reduce the release of excitatory amino acid neurotransmitters and inhibit epilepsy via an interaction with the dizocilpine binding site of NMDA receptor, which is in accord with the theory that the glutamic acid receptor antagonists can inhibit epilepsy. The combination of ineffective dose of dizocilpine with phenobarbital can significantly shorten ADD and Racine's stage, which suggests that a synergism exists between this two drugs. It is known that phenobarbital can hyperpolarize cell membrane via increasing Cl^- influx and prolonging the open-time of Cl^- channel and blocking the subtype of glutamate receptor and Ca^{2+} channel. Non-competitive antagonists are of the characteristic of agonists-dependent and voltage-dependent, which can not antagonize without enough agonists and fairly low voltage. Hyperpolarization would increase the binding ability of dizocilpine with the binding site, which might be the cause of the synergism of dizocilpine and phenobarbital.

Dizocilpine blocks NMDA receptors via inhibiting Ca^{2+} influx. NMDA receptors form complex by coupling with Ca^{2+} channels. Nicardipine is a voltage-dependent antagonist, which has been shown to inhibit the amygdala kindling in rats[8]. Dizocilpine and nicardipine can antagonize epilepsy from different direction. It has been shown that combination of dizocilpine with nicardipine can significantly inhibit ADD.

Valproate can antagonize epilepsy by activating glutamic acid decarboxylase (GAD) and inhibiting GABA metabolic enzyme and increasing GABA content in brain. Combination of ineffective-dose of dizocilpine and valproate can remarkablely shorten ADD and decrease Racine's stage, which suggests that the two drugs act

similarly in GABA system. SCZ is a GAD inhibitor which can induce seizure by decreasing GABA. Dizocilpine can significantly inhibit convulsion, indicating that the effect of dizocilpine is related to potentiation of the GABA function. The inhibition mechanism is related to the blockade of the voltage-operated calcium channels and the enhancement of the action of GABAergic system in the brain.

The release of neurotransmitters requires Ca^{2+}. The relation between dizocilpine and Ca^{2+} channel or GABA system needs further study.

Dizocilpine inhibits the seizure of the amygdala kindling. It can improve the antiepileptic activity of phenobarbital, valproate and nicardipine. Its combination with other drugs may provide a new method of treating epilepsy.

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