2 Yang KT, Hsu CK, T'ung KF. Scopolamine in emergency treatment of respiratory failure in very severe encephalitis B. *Chin Med J* 1973; **53**: 283
3 Pan LH, Zhan WZ, Zhang JR. Relation between high-frequency oscillation in phrenic discharge and duration of inspiration. *Acta Physiol Sin* 1982; **34**: 49
4 Hammer R, Berrie CP, Birdsall NJM, Burgen ASV, Hulme EC. Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* 1980; **283**: 90
5 Bian CF, Xing SH, Shao LN, Qin W. Central inhibitory effects of pirenzepine. *Acta Pharmacol Sin* 1987; **8**: 396
6 Giraldo E, Hammer R, Landisky H. Distribution of muscarinic receptor subtypes in rat brain as determined in binding studies with AF-DX 116 and pirenzepine. *Life Sci* 1987; **40**: 833
7 Zheng JL, Bian CF. Analysis of muscarinic receptor subtypes in rat brain stem. *Acta Acad Med Xuzhou* 1991; **11**: 5

抗胆碱药对兔膈神经放电的影响

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提要　于清醒、肌松、双侧迷走神经切断的兔，记录膈神经放电，小脑延髓池注射药物．东莨菪碱 0.5 mg·kg^{-1} 和哌仑西平 0.5 mg·kg^{-1} 使膈神经放电频率减少，电压降低($P<0.05$)，阿托品 0.05 mg·kg^{-1} 和 AF-DX 116 0.1 mg·kg^{-1} 使放电频率增加，电压增大($P<0.01$)，结果显示东莨菪碱抑制呼吸中枢，可能与其阻断 M_1 受体有关，阿托品的呼吸中枢兴奋作用，可能与其阻断 M_2 受体有关．

关键词　呼吸中枢；膈神经；电生理；东莨菪碱；哌仑西平；阿托品；苯并二氮䓬类

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Effects of nimodipine on *l*-glutamate-induced seizures and Ca^{2+} influx in hippocampus in freely moving rats

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ABSTRACT　Seizure (EEG) was studied in rats unilaterally injected in the dorsal hippocampus with *l*-glutamate (Glu). Extracellular Ca^{2+} content [$(Ca^{2+})_e$] in the injected area was assessed by brain microdialysis coupled to automatic atomic absorption spectrophotometry. In this experimental epileptic model, an inhibition of Glu-stimulated epileptic activity and a fall in $(Ca^{2+})_e$ by nimodipine (Nim, 100 μg·kg^{-1}) were seen. The spike- and wave-burst frequency was reduced from 30 to 5 bursts·min^{-1} ($P<0.01$, $n=8$). Nim 25 and 50 μg·kg^{-1}, without anticonvulsant activity, did not prevent the drop in $(Ca^{2+})_e$. These results indicate that Nim exerts an antiepileptic effect on Glu-induced epilepsy. The mechanisms may be involved in blocking Ca^{2+} influx into neurons.

KEY WORDS　calcium; nimodipine; spectrophotometry; epilepsy

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Ca^{2+} influx into neurons seems to play an important role in excitatory amino acids-induced epileptic activity[1]. Experimental studies exploring antiepileptic activity of calcium antagonists revealed anticonvulsive properties of flunarizine[2] and verapamil[3]. Nimodipine (Nim), so far studied for its effects, was only restricted to the cerebral

vessels[4]. The aim of this work was to investigate the actions of Nim on *L*-glutamate (Glu)-induced seizures and Ca^{2+} influx in hippocampus to evaluate its antiepileptic properties.

MATERIALS AND METHODS

EEG recording and intrahippocampal injection[5] Sprague-Dawley rats, ♂, (220 ± SD 19 g) were placed in a Stoelting stereotoxic apparatus under chloral hydrate (360 mg • kg^{-1} ip) anesthesia. The fiber with attached electrode and cannula guide was set up as previously describe and was unilaterally implanted into the dorsal hippocampus[5]. EEG recording (4-channel EEG polygraph, model B8P, Battaglia Rangoni, Bologna, Italy) was made for at least 30 min to assess the spontaneous EEG pattern. Glu (250 mmol • L^{-1} in sterile water) 1 μl was injected (60 s) through needle which extended 3.0 mm below the guide cannula. EEG was continuously recorded for at least 3 h after Glu infusion.

***In vivo* perfusion and Ca^{2+} measurements**[5] The unilaterally implanted fiber was perfused at a rate of 2 μl • min^{-1} with nominally Ca^{2+} free Krebs-Ringer bicarbonate solution (mmol • L^{-1}): NaCl 122; KCl 3; $MgSO_4$ 1.2; KH_2PO_4 0.4; $NaHCO_3$ 25; pH 7.4 and 10-min perfusates were collected. Ca^{2+} content in the perfusate was determined by automatic atomic absorption spectrophotometry (Model GAC 908 AA, Shimadzu, Japan).

Histological verification The position of the fiber in the dorsal hippocampus was verified by histological technique on 40-μm black ink stained sections. Only rats with correct position were counted.

Nim treatment Nim, made by Tianjing Institute of Pharmaceuticals, was dissolved in dimethyl sulfoxide (DMSO, final concentration 1%). First, Nim was administered iv in 6 rats at increasing doses of 25 ($n=2$), 50 ($n=2$), and 100 ($n=2$) μg • kg^{-1} to study the direct effects of the Nim on the normal EEG. Then, in a group of rats with seizure induced by Glu (Sigma Chemicals Co, St Louis MO USA), Nim was injected iv at the doses of 25 (8 rats), 50 (8 rats), and 100 (8 rats) μg • kg^{-1} 20 min after the application of Glu 1 μl (250 mmol • L^{-1}, intrahippocampal injection). The EEG and extracellular Ca^{2+} [$(Ca^{2+})_e$] were compared with those of control group (8 rats) to which DMSO 1% was injected iv.

Statistical analysis In EEG numbers of spike- and wave-bursts per minute were evaluated on ipsilateral and contralateral hemispheres. Ca^{2+} concentration (μmol • L^{-1} perfusate) after drugs treatment was compared with that in control. All values were expressed as $\bar{x} \pm$ SD. The data were statistical analyzed by two-tailed *t* test.

RESULTS

Glu-induced seizure All the rats ($n=24$) unilaterally injected in the dorsal hippocampus with Glu 1 μl (250 mmol • L^{-1}) developed epileptic activity (Fig 1). This activity consisted of the presence of spike- and wave-bursts. Quantitative evaluation of seizure activity showed a typical latency to onset of 24 min. Maximum activity was then observed at 40th min (30 ± 5 bursts • min^{-1}); this pattern

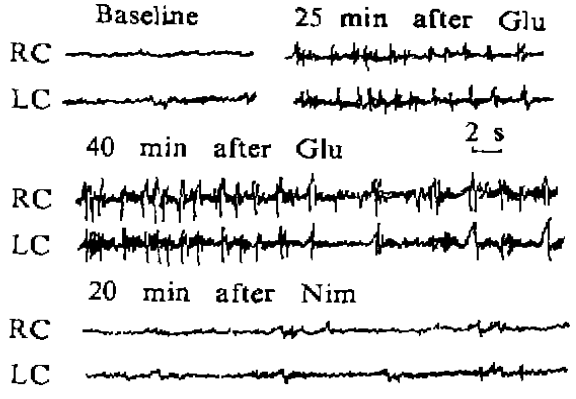

Fig 1. Effects of Nim on Glu-induced seizure (EEG).

persisted substantially unmodified until 90th min of observation. In the contralateral hemisphere lead, the same type EEG pattern was observed, characterized, however, by a later (at 33th min) appearance and by a slower reaching of the maximum point of activity (50th min). The rats showed episodes of tonic rotation of the head, associated with chewing movements.

Glu-induced $(Ca^{2+})_e$ decrease in the injected hippocampus $(Ca^{2+})_e$ progressively decreased from 90.1 ± 10.2 (control) to 61.2 ± 5.8 $\mu mol \cdot L^{-1}$ at 25 min from injection (seizure onset). Between 25 and 90 min (maximal seizure activity) $(Ca^{2+})_e$ was reduced to 48.5 ± 6.2 $\mu mol \cdot L^{-1}$ and during 120 min (seizure-free time), it gradually returned to control (Fig 2).

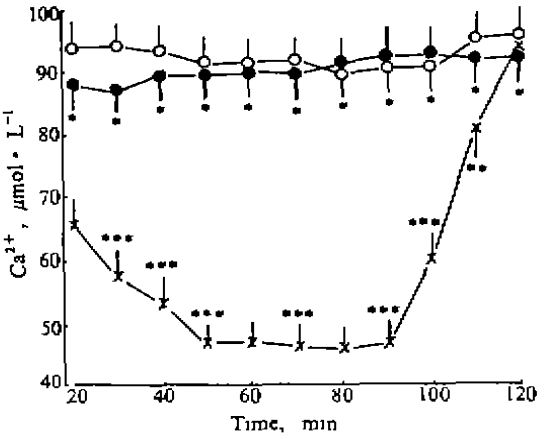

Fig 2. $(Ca^{2+})_e$ in dorsal hippocampus after Glu (×) and Nim (●), Control (○). $n=8$, $\bar{x} \pm SD$, $^*P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

Effects of Nim on the normal EEG pattern Nim did not produce changes in the normal EEG tracing in the rats. The maximum spike activity was less than 5 bursts · min^{-1}.

Effects of Nim on Glu-induced seizure Nim 25 and 50 $\mu g \cdot kg^{-1}$ did not produce significant changes in the epileptic activity induced by Glu 1 μl (250 mmol · L^{-1}). In the rat group treated with Nim 100 $\mu g \cdot kg^{-1}$, a significant reduction of epileptic activity was observed in the ipsilateral hemisphere leads 20 min after the administration (Fig 3A). This effect was even more evident in the successive observation periods, until reaching an almost complete suppression of epileptic activity from the 60th min. In the contralateral hemisphere leads, a significant reduction (Fig 3B) of burst frequency was observed already from 15 min after Nim administration, this effect increased until reaching an almost total disappearence of the epileptic activity 40 min after the injection.

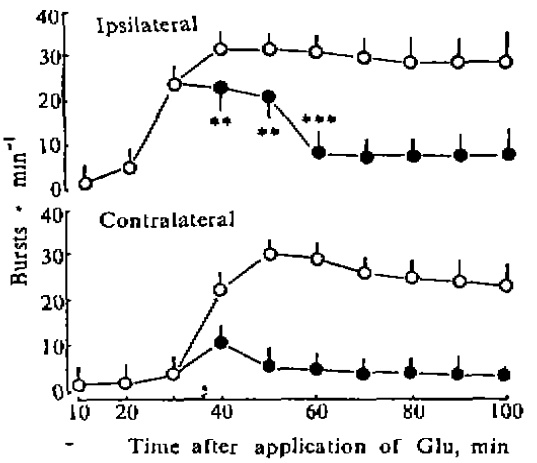

Fig 3. Effect of Nim (●) on Glu-induced ipsilateral (A) and contralateral (B) burst activity (○). $n=8$, $\bar{x} \pm SD$, $^{**}P<0.05$, $^{***}P<0.01$.

Effects of Nim on the reduction in $(Ca^{2+})_e$ caused by Glu Nim (100 $\mu g \cdot kg^{-1}$) antagonized the decrease in $(Ca^{2+})_e$ induced by Glu 1 μl (250 mmol · L^{-1}). Inhibition% was 95.4 ± 9.2 at 30th min. But at the doses of 25 and 50 $\mu g \cdot kg^{-1}$ did not modify drop in $(Ca^{2+})_e$ (Fig 2).

DISCUSSION

The results of our study showed that Nim, at the dose of 100 $\mu g \cdot kg^{-1}$, exerted an

anticpileptic effect in the Glu-induced epilepsy. It was worth noting that this effect developed earlier in the contralateral hemisphere. This would indicate an action of Nim not only on the epileptic focus level, but also and earlier on the diffusion of this activity to the cerebral areas of the opposite hemisphere.

The present findings also indicated that Ca^{2+} entry into neurons played a role in the genesis of focal seizures as the seizures associated with $(Ca^{2+})_e$ drop. The ability of Nim which protected against epilepsy to prevent the $(Ca^{2+})_e$ fall further suggested that Ca^{2+} influx induced by Glu was closely involved in the mechanisms underlying seizure occurence.

A previous experimental study on rats[4] has shown that Nim (at dose of 100 $\mu g \cdot kg^{-1}$), although reducing the mean arterial presure, produced cerebral blood flow increase through a selective and marked dilatatory effect on the cerebral vessels. For this reason, our experiments would not cause an impairment of the cerebral perfusion and the possibility of a clinical use of Nim in the field of epilepsy.

REFERENCES

1 Vezzani A, Wu HQ, Angelico P, Stasi MA, Samanin R. Quinolinic acid-induced seizures, but not nerve cell death, are associated with extracellular Ca^{2+} decrease assessed in the hippocampus by brain dialysis. *Brain Res* 1988; **454**: 289

2 Wauquier A, Ashton D, Melis W. Behavioral analysis of amygdaloid kindling in beagle dogs and the effects of clonazepam, diazepam, phenobarbital, diphenylhydantoin, and flunarizine on seizure manifestation. *Exp Neurol* 1979; **64**: 579

3 Walden J, Speckmann E-J, Witte OW. Suppression of focal epileptiform discharges by intraventricular perfusion of a calcium antagonist. *Electroencephalogr Clin Neurophysiol* 1985; **61**: 299

4 Haws CW, Heistad DD. Effects of nimodipine on cerebral vasoconstrictor responses. *Am J Physiol* 1984; **247**: H170

5 Lu YM, Liu GQ. Effects of (−)-daurisoline on quinolinic acid-induced Ca^{2+} influx in neurons in freely moving rats. *Acta Pharmacol Sin* 1991; **12** (in press)

尼莫地平对自由活动大鼠谷氨酸致癫痫和海马神经元钙内流的影响

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提要 观察尼莫地平(Nim)对大鼠单侧海马注射谷氨酸(Glu)致癫痫的影响. 结果表明 Nim (100 $\mu g \cdot kg^{-1}$ iv)显著抑制 Glu 引起的癫痫样脑电活动(最大高幅放电频率从 30 次/分降到 5 次/分，$n=8$，$P<0.01$). 同时抑制 Glu 引起的$(Ca^{2+})_e$ 内流. 当 Nim iv 分别为 25 和 50 $\mu g \cdot kg^{-1}$ 时，不影响癫痫样脑电活动，也不拮抗海马神经元$(Ca^{2+})_e$ 内流. 因此认为 Nim 具有抗 Glu 致癫痫作用，其机制可能与阻断神经元$(Ca^{2+})_e$ 内流有关.

关键词 钙；尼莫地平；分光光度测定法；癫痫