experiments showed that *l*-daurisoline acted immediately on NMDA-operated calcium channel, but not on NMDA receptor and further supported our previous findings[7,8] that *l*-daurisoline potently blocked voltage-operated or receptor-operated $Ca^{2+}$ channels.

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## 左旋蝙蝠葛苏林碱对自由活动大鼠喹啉酸引起海马神经元钙内流的影响


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**提要** 观察自由活动大鼠海马神经元外 $Ca^{2+}$ 浓度 $(Ca^{2+})_e$ 同时记录脑电活动. 海马内注射 *N*-甲基-*D*-门冬氨酸(NMDA)受体激动剂喹啉酸 156 nmol, 1 h 后，注射部位由于 $(Ca^{2+})_e$ 内流，$(Ca^{2+})_e$ 下降 48 ± 5% 伴随两侧皮层脑电异常. 左旋蝙蝠葛苏林碱(*l*-Dsl)拮抗喹啉酸引起的 $(Ca^{2+})_e$ 下降，不影响异常脑电. 提示，*l*-Dsl 阻断 NMDA 操纵 $Ca^{2+}$ 通道，不拮抗 NMDA 受体.



**关键词** 蝙蝠葛苏林碱；喹啉酸；钙通道阻滞剂；海马；惊厥



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# Effect of intrahippocampal quinolinic acid infusion on the amygdala kindling in rat[1]


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**ABSTRACT** The effect of intrahippocampal infusion of quinolinic acid (Quin), an endogenous excitatory amino acid, was studied on the amygdala kindling. Quin 120 nmol injected intrahippocampally 2 wk prior to the beginning of amygdala kindling significantly not only produced dorsal hippocampal pyramidal and granule cell loss but also decreased the number of stimuli to trigger the stage 5 seizures of amygdala kindling. In kindled rats, intrahippocampal 20 nmol Quin infusion fully inhibited the stage 5 of amygdala-kindled seizures. The inhibitory effect of Quin was antagonized by *dl*-2-amino-7-phosphonoheptanoic acid, a selective



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antagonist of *N*–methyl–*D*–aspartate (NMDA) type receptors. The results suggest that NMDA–type receptors in the hippocampus may play a role in the control of the seizure threshold in the amygdala.

**KEY WORDS** quinolinic acids; kindling (neurology); hippocampus; amygdaloid body

Kindling is a phenomenon of epileptogenesis which develops over time in response to repeated electric stimulation of various brain regions[1]. *N*–methyl–*D*–aspartate (NMDA) subtype of excitatory amino acid (EAA) receptors is involved in the induction of the long–lasting alterations in neuronal excitability and in kindling phenomenon[2]. Electrophysiological findings on hippocampal slices showed that NMDA–type receptors become involved in synaptic transmission after kindling of the amygdala or hippocampus[3]. The reciprocal connection exists between amygdala and hippocampus through direct and indirect projections[4]. However, it is not clear whether NMDA–type receptors in hippocampus are involved in the seizure threshold of amygdala kindling. Thus, we observed the effect of intrahippocampal infusion of quinolinic acid (Quin), an endogenous NMDA receptor agonist[5], on the development and maintenance of amygdala kindling.

## MATERIALS AND METHODS

Male Sprague–Dawley rats, weighing 275 ± SD 25 g, were used. The rats were implanted with a bipolar electrode stereotaxically aimed at the left basolateral amygdala (A 3.8, L 4.2, H −3.2 mm)[6] under equitensin anesthesia (3 ml · $kg^{-1}$) according to our previous methods[7]. The bipolar electrodes were constructed of twisted nichel wire, 170–μm in diameter, and insulated with teflon except at the cut tips. Holes were made in the calvarium to place the screw electrodes over the parietal cortex bilaterally. All electrodes were attached to a multipin socket and secured to the skull by acrylic dental cement. For microinjection into the left dorsal hippocampus (A 3.3, L 3.2, H 2.5 mm)[6], guide cannula (22 gauge stainless steel tube) was chronically implanted and was positioned on the top of the dura for drug infusion. After surgery, rats were housed individually and allowed to recover.

Electric stimulation consisted of a 1–s train of 150–250 μA, 60 Hz biphasic square waves each 1 ms in duration and was delivered daily to the left amygdala from a DC stimulator through a constant current output[7]. The electroencephalogram (EEG) was recorded before and after each stimulation between the tips of the bipolar electrode. The measurement of the seizure activity was (A) an assessment of behavioral severity of convulsions according to the ranking scale defined by Racine[1]: 0–no behavioral response to amygdala stimulation; 1–facial clonus; 2–head nodding; 3–forelimb clonus; 4–rearing; and 5–rearing and falling and (B) the EEG afterdischarge (AD) duration. The rats were considered 'kindled' when stage 5 seizures were observed after stimulation of the amygdala.

Quin and *dl*–2–amino–7–phosphonoheptanoic acid (APH) (purchased from Sigma Chemical Co, USA) were dissolved in phosphate buffer 0.01 mol · $L^{-1}$ solution (PBS) (pH = 7.4). The volume for dorsal intrahippocampal infusion was 1 μl. In kindling rats, Quin 120 nmol was slowly infused into left dorsal hippocampus 2 wk prior to the beginning of the amygdala stimulation. In kindled rats, Quin 20 nmol was slowly injected into left dorsal hippocampus 30 min prior to the beginning of the amygdala stimulation.

Upon completion of the experiments, histological examination was performed on thionin–stained, 40–μm cryostat sections. The extent of neuronal degeneration induced

by Quin and the placement of electrodes and cannula were histologically verified in all rats. The data were discarded when implanted position was inaccurate. The significance was tested by group comparison of $t$ test.

## RESULTS

**Effects of intrahippocampal Quin infusion on development of amygdala kindling** PBS 0.01 mol · $L^{-1}$ (1 $\mu$l) or Quin 120 nmol was slowly infused into left dorsal hippocampus 2 wk prior to the beginning of amygdala stimulation. In PBS-control rats, 15.2 ± 2.8 stimuli were needed to trigger the first stage 5 seizures. The number of stimuli to reach the first stage 5 seizures in Quin intrahippocampally injected group was 10.4 ± 1.9 ($P < 0.05$). The AD durations of the 2 groups were not notably different (Tab 1).

**Tab 1. Effects of intrahippocampal quinolinic acid (Quin) 120 nmol infusion on the development of amygdala kindling.** $\bar{x} \pm$ SD. $^{*}P > 0.05$, $^{**}P < 0.05$ *vs* **phosphate buffer solution (PBS) group.**

| | $n$ | Number of stimulations to reacth the first stage 5 | Duration of after-discharge of stage 5 (s) |
|---|---|---|---|
| PBS | 10 | 15.2 ± 2.8 | 59.0 ± 5.1 |
| Quin | 7 | 10.4 ± 1.9** | 49.2 ± 13.5* |

The histological examination showed that ipsilateral and contralateral hippocampal structures had little difference in control rats. Almost complete degeneration of dorsal hippocampal pyramidal and granule cells were seen in the Quin-injected site. The contralateral hippocampus was not influenced.

**Effect of intrahippocampal Quin infusion on amygdala-kindled seizures** In the kindled rats, obvious EEG and behavioral alterations were not seen when Quin 20 nmol was injected 30 min prior to amygdala stimulation. This dose of Quin fully blocked the stage 5 of amygdala-kindled seizures. The inhibitory effect of Quin was abolished by APH 8 nmol. The AD duration was not altered (Tab 2). Histological examination of hippocampus did not reveal obvious difference between control rats and Quin-injected rats.

**Tab 2. Effects of intrahippocampal quinolinic acid (Quin) infusion on the amygdala-kindled seizures and the antagonism of *dl*-2-amino-7-phosphonoheptanoic acid (APH).** $\bar{x} \pm$ SD. $^{*}P > 0.05$, *vs* **phosphate buffer solution (PBS) group.**

| | Stage 5 seizure | Duration of afterdischarge (s) |
|---|---|---|
| PBS | 7 / 7 rats | 43.0 ± 21.9 |
| Quin (20 nmol) | 0 / 7 rats | 83.2 ± 28.3* |
| Quin (20 nmol) + APH (8 nmol) | 6 / 7 rats | 29.4 ± 8.5* |

## DISCUSSION

Quin is an endogenous neurotoxin and a potent convulsant acting NMDA type receptors when it is injected into the rat hippocampus, amygdala, and cortex[5]. Obvious electrical encephalographic and neuropathological changes appeared when Quin's icv concentration was more than 120 nmol. Low concentration of Quin (< 30 nmol) activated central neurones but did not induce neuropathological alterations[8]. So, 2 doses of Quin were chosen for our studies to investigate the modulatory effect of hippocampus in amygdala kindling and kindled seizures. Present paper showd that Quin 20 nmol injected into the hippocampus prevented amygdala-kindled seizures in rats and the inhibitory action of Quin was abolished by selective NMDA receptor blocker APH. Hippocampal cell loss induced by Quin 120 nmol facilitates the development of amygdala kindling. Electrophysiological and biological results suggest that the endogenous tryptophan metabolite Quin acts selectively on the NMDA type of excitatory amino acid receptors to produce excitation and loss of

central neurons[9]. Quin-induced seizures and hippocampal nerve cell loss can be antagonized by APH. These findings suggest that NMDA receptors in the hippocampus participate in amygdala kindling.

Much evidence indicates that excitatory amino acid receptor blockers administered centrally or peripherally can inhibit the amygdala-kindled seizures in mice, rat, and rabbit[2]. But Cavalheiro and Turski reported that intrastriatal NMDA prevented amygdala-kindled seizures and shortened AD duration in rats[10]. We found that Quin intrahippocampally infused in low dose inhibited amygdala-kindled seizures. Both facilitatory and inhibitory interactions between the amygdala and hippocampus are possibly existed in kindling phenomenon (personal communication from Dr. Ron J Racine, Oct 5, 1990). The reciprocal connection exists between amygdala and hippocampus through direct and indirect projections[4]. Most of the fibers in the major afferent and intrinsic excitatory projection systems in the hippocampal formation might be glutamate fibers. The hippocampus influences other brain regions by means of extensive cortical and subcortical projections[11]. We think that NMDA receptor activation induced by Quin at low dose in hippocampus inhibited the amygdaloid neuronal activity. Amygdaloid neurones were deinhibited when hippocampal nerve cells were degenerated by Quin injected at high dose. Our results suggest that hippocampus plays a role in the control of seizure threshold of amygdala kindling.

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## 海马内注射喹啉酸对大鼠杏仁核点燃效应的影响

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**提要**　海马内注射 120 nmol 的喹啉酸(Quin)不仅引起明显的海马锥体细胞和颗粒细胞降解，而且缩短杏仁核点燃效应五期反应的出现时间．海马内注射 20 nmol 的 Quin 可完全抑制杏仁核点燃效应的五期反应．*d,l*-2-氨基-7-磷酸庚酸(APH)可对抗 Quin 的抑制作用．结果表明，海马的 NMDA 受体在杏仁核点燃过程中对发作阈值可能有重要的调控作用．

**关键词**　喹啉酸类；诱发(神经病学)；海马；杏仁核