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Effects of *l*—daurisoline on quinolinic acid—induced Ca^{2+} influx in hippocampus neurons in freely moving rats[1]

LU You—Ming, LIU Guo—Qing
(*Department of Pharmacology, China Pharmaceutical University, Nanjing 210049, China*)

ABSTRACT In freely moving rats, hippocampus neuronal extracellular calcium concentration $(Ca^{2+})_e$ and seizures were investigated. Application of quinolinic acid 156 nmol (exciting *N*—methyl—*D*—aspartate receptor, NMDA) to dorsal hippocampus elicited a decrease in $(Ca^{2+})_e$ by 48 ± 5% in the infusion area and produced a characteristic abnormal EEG. *l*—Daurisoline dramatically prevented the reduction in $(Ca^{2+})_e$, but not seizures (EEG). The results suggest that NMDA—operated calcium channels, but not NMDA—receptors, are involved in the effects of *l*—daurisoline on Ca^{2+} influx observed.

KEY WORDS daurisoline; quinolinic acids; calcium channel blockers; hippocampus; seizures

Quinolinic acid (Quin), an excitotoxic convulsant brain metabolite[1], preferentially acts on *N*—methyl—*D*—aspartate (NMDA) receptors. The activation of NMDA receptors causes Ca^{2+} entry into the neurons through the NMDA—operated Ca^{2+} channels[2,3]. Electrophysiological studies have shown that Ca^{2+} influx often precedes neuronal burst response and the onset of seizures[4]. It has been suggested that calcium has a significant role in the epileptogenic effects of Quin.

In the present study, we have combined the dialysis fiber with recording electrodes to collect brain perfusates, while simultaneously recording EEG activity. Using this technique, we examined the effects of *l*—daurisoiine on the changes of the local $(Ca^{2+})_e$ and EEG induced by Quin.

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MATERIALS AND METHODS

Implantation procedure Sprague—Dawley rats, ♂, (233 ± SD 19 g) were placed in a Stoelting stereotaxic apparatus under chloral hydrate (360 mg · kg^{-1}) anesthesis. A single fiber (molecular cut off 15 000; internal diameter 220 μm; external diameter 310 μm) was straightened with a fine wire inserted through its lumen and then bent into a loop. Fast—drying glue was spread over the surface of the fiber except the 4—mm portion at the tip of the loop to allow exchange with the tissue. An injection cannula guide tube (22 gauge) was fixed to the side of the fiber and the bottom of the guide tube was placed 3.0 mm above the loop (Fig 1).

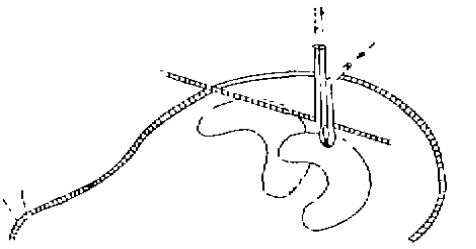

Fig 1. Dialysis fiber implanted unilaterally into dorsal hippocampus in rat was connected to a cannula guide tube (dotted arrow) for intrahippocampal injection of drugs. Solid arrows = inlet and outlet portions of the perfusion assembly. [The 4—mm section of the fiber open for dialysis is indicated at the tip of the loop (black portion)].

The fiber assembly with attached cannula guide was unilaterally implanted into the dorsal hippocampus. The coordinates for implantation were 3.5 mm posterior to bregma, 2.3 mm lateral to the medline and 3.0 mm below dura. Stainless steel screw

electrods were placed bilaterally over the sensory motor cortex and a ground lead screw over the nasal sinus. The electrodes were connected to a multipin socket and together with the fiber units, and secured to the skull by acrylic dental cement.

EEG recording and intrahippocampal injection The EEG was recorded (8-channel EEG Polygraph, Model SMIC ND-82B) for at least 30 min to assess the spontaneous EEG pattern. Quin was dissolved in NaOH (1 mol · L^{-1}); *l*-daurisoline was dissolved in HCl (1 mol · L^{-1}). The solution were neutralized (pH = 7.4) and brought to the final volume with phosphate-buffer saline (0.1 mol · L^{-1}). One μl of the solution was slowly injected (60 s) through needle which extended 3.0 mm below the guide cannula in order to deliver the drug at the site of perfusion. The needle was left in place for another minute and then removed.

EEG was recorded continuously for at least 180 min after drug infusion. The EEG recording was analysed visually to detect any activity different from control. EEG consisted in a simultaneous occurrence of following seizure activities: high frequency multispike complexes and high voltage synchronized spiking 30 min after Quin injection (Fig 2).

The estimation of Ca^{2+} recovery *in vitro* For estimation of Ca^{2+} recovery through the fiber membrane, the dialysis fiber was perfused *in vitro* at 2 μl · min^{-1} with nominally Ca^{2+}-free Krebs-Ringer bicarbonate (KRB) (in mmol · L^{-1}) NaCl 122; KCl 3; $MgSO_4$ 1.2; KH_2PO_4 0.4; $NaHCO_3$ 25 and placed in a solution of KRB containing $CaCl_2$ (1 mmol · L^{-1}). After 30 min washout to reach equilibration between the lumen of the fiber and the external solution, six 20-min samples were collected. The average recovery of Ca^{2+} was determined using 3 different fiber units. By dialysis 12.0 ± 0.6% of Ca^{2+} was recovered, expressing the mean Ca^{2+} concentration in the perfusate as a percentage of the concentration of the solution outside the tube. The results were calculated as Ca^{2+} concentration per 20 min fraction. After 60 min perfusion in KRB+$CaCl_2$ (1 mmol · L^{-1}) the fiber was placed in KRB solution containing $CaCl_2$ (0.5 mmol · L^{-1}) for 30 min and then again in KRB+$CaCl_2$ (1 mmol · L^{-1}) for another 40 min. A 50% decrease or increase in the Ca^{2+} concentration of the external medium was proportionally reflected in the Ca^{2+} content of the perfusate in the second 20-min sample collected after changing the solution.

***In vivo* perfusion** On the day after surgery the unilaterally implanted fibers were perfused at a rate of 2 μl · min^{-1} with KRB (pH = 7.4), nominally Ca^{2+}-free, and 20-min perfusates were transported to small collection tubes via short Teflon tubings attached to the fiber outlet. To ensure that a stable Ca^{2+} content baseline was established, samples were collected for 60 min prior to the intrahippocampal injection. Following the drug or phosphate-buffer saline (0.1 mol · L^{-1}, control) injection, samples were taken for a minimum of 3 h. Ca^{2+} was measured by Automatic Atomic Absorption Spectrophotometer (model GBC 908 AA, Shimadzu, Japan). The rat brains were histologically examined. Only the rats with correct positioning were used as results.

Chemical reagents Quin and verapamil were purchased from Sigma Chemical Co. *l*-Daurisoline was isolated and purified from the roots of *Menispermum dauricum* by *Department of Pharmaceutical Chemistry, China Pharmaceutical University*.

RESULTS

Fig 2 showed the EEG activity of a freely moving rat unilaterally injected in the dorsal hippocampus with Quin 156 nmol. Ictal

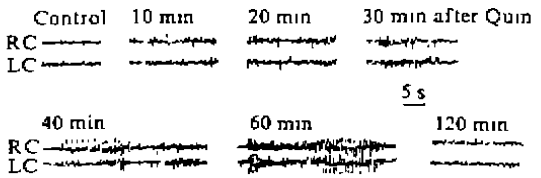

Fig 2. A typical EEG tracing seizure activity and synchronized spiking of a rat injected Quin 156 nmol in left hippocampus. LC = Left cortex; RC = right cortex.

episodes were characterized by abnormal EEG patterns in right and left cortex leads. Pretreatment with *l*–daurisoline (10 $\mu g \cdot \mu l^{-1}$) or verapamil (15 $\mu g \cdot \mu l^{-1}$) did not modify the Quin–induced abnormal EEG. Episodes lasted about 2 h, followed by one seizure–free hour in which synchronized spiking sometimes appeared.

Fig 3 sets out the effect of Quin 156 nmol on the $(Ca^{2+})_e$ in the infusion area in the hippocampus. $(Ca^{2+})_e$ progressively decreased to 36.8% below control 40 min after injection (seizure onset). Between 60 and 80 min (maximal seizure activity) Ca^{2+} was reduced by about 48.4% and after 120 min (seizure–free time) it gradually returned to control.

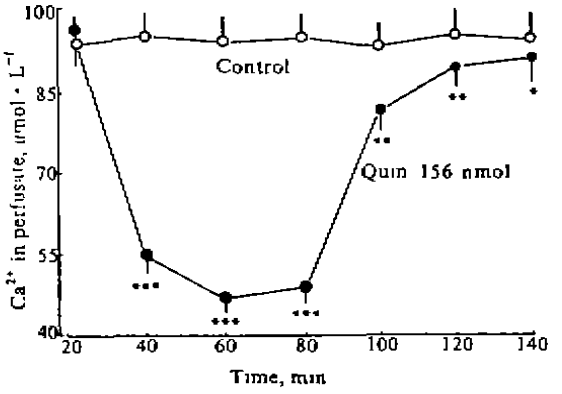

Fig 3. Extracellular Ca^{2+} changes induced by Quin injected into left hippocampus. $n=7$ rats, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

l–Daurisoline (5 or 10 μg), 30 min after intrahippocampal injection, antagonized the decrease in $(Ca^{2+})_e$ induced by Quin (156 nmol) (Tab 1). Inhibitory rates were 54 ± 8% and 96 ± 11%, respectively. Verapamil (50 μg) was not active.

Tab 1. Effects of *l*–daurisoline (Dsl) and verapamil (Ver) on extracellular Ca^{2+} decrease 60 min after intrahippocampal injection of quinolinic acid (156 nmol) in freely moving rats. $n=9$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{***}P<0.01$ *vs* control.

Drug (μg)	Ca^{2+} concentration ($\mu mol \cdot L^{-1}$) Baseline	With Quin	Decrease %
Control	95 ± 11	49 ± 5	48 ± 6
Dsl (5)	94 ± 10	73 ± 8	22 ± 2***
(10)	95 ± 10	93 ± 11	2.0 ± 0.1***
Ver (50)	96 ± 12	48 ± 4	50 ± 5*

DISCUSSION

The results showed that significant changes in $(Ca^{2+})_e$ occurred in the injected hippocampus with amounts of Quin causing seizures in all rats. A comparative analysis of Ca^{2+} content in brain perfusate and EEG activity at various times after Quin administration indicated a close temporal correlation between Ca^{2+} changes in injected area and development of seizure activity. Moreover, the selective NMDA receptor antagonist *D*–(–)–2–amino–7–phosphonoheptanoic acid (AP–7)[5] at a dose shown to block the excitotoxic action of Quin, prevented the fall in $(Ca^{2+})_e$ indicating that this effect was receptor mediated.

In the present study, *l*–daurisoline dramatically antagonized Ca^{2+} influx induced by Quin, but failed to modify abnormal EEG. This functional study of *l*–daurisoline is consistent with the investigation of Mg^{2+}, a calcium channel blocker, inhibiting the changes in the $(Ca^{2+})_e$ induced by Quin without anticonvulsant activity[6]. The

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

鲁友明、刘国卿

(中国药科大学药理教研室，南京 210009，中国)

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]

PING Han-Xian, LIU Guo-Qing, XIE Lin, WU Hui-Qiu (*Department of Pharmacology, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China*)

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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