Effects of Ca^{2+} antagonists on glutamate release and Ca^{2+} influx in the hippocampus with *in vivo* intracerebral microdialysis

¹Y. Ming Lu, *J. Tian Zhang, F. Qing Zhao & Y. Fi Qin

Department of Molecular Pharmacology, Naval Neurobiology Research Centre, China Naval Medical College, Nanjing, 210049, China and *Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing 100050, China

1 The extracellular glutamate content and Ca^{2+} level *in vivo* in rat hippocampus were measured by brain microdialysis following administration of two depolarizing agents (veratridine, KCl) and quinolinic acid (Quin).

2 The two depolarizing agents increased the extracellular glutamate level (to between 280 and 320% basal) and decreased the extracellular Ca^{2+} content (to 48% of basal). However, Quin did not change the glutamate level but decreased the Ca^{2+} content.

3 The effects of Ca^{2+} antagonists on the changes of glutamate and Ca^{2+} level were evaluated in this experimental model. At a dose of 0.5 mg kg^{-1} , i.v., nimodipine (L-type channel blocker) did not produce significant changes in the stimulated-glutamate release. A statistically significant inhibition of Ca^{2+} influx was observed at a dose of 0.05 mg kg^{-1} . In contrast, in those animals receiving the N-type Ca^{2+} antagonist, daurisolone (0.1 , 1 or 5 mg kg^{-1} , i.v.), a potent attenuation of both glutamate release and Ca^{2+} influx was found.

4 We propose that the pharmacological properties of Ca^{2+} influx and of neurotransmitter release differ and that nimodipine-sensitive L-type channels may not be very common in nerve terminals but are localized in cell soma. Daurisolone-sensitive N-type channels in nerve terminals have a much greater influence on excitatory amino acid release.

Keywords: Glutamate release; Ca^{2+} influx; multiple Ca^{2+} channels; depolarizing agents; quinolinic acid; nimodipine; daurisolone; intracerebral microdialysis

Introduction

The indispensable role of Ca^{2+} in the release of neurotransmitters is well established. Experimental evidence from various sources has indicated that neurones and other excitable cells may possess several types of voltage-sensitive calcium channels (VSCC) (Carbone & Lux, 1984; Nowicky *et al.*, 1985a). These channels can be distinguished by their biophysical characteristics and also by their sensitivity to drugs, hormones, and toxins (Triggle & Rampe, 1989). One type of VSCC has been characterized in several different tissues by its sensitivity to dihydropyridine drugs (Freedman & Miller, 1984; Miller & Freeman, 1984; Nowicky *et al.*, 1985b). A subject of great current interest is the elucidation of the pharmacological properties of both dihydropyridine-sensitive (L-type channel) and dihydropyridine-insensitive (N-type channels) VSCC. In neurones, a major function of VSCC is to provide Ca^{2+} for triggering the release of neurotransmitters (stimulus-secretion coupling). The evoked release of neurotransmitters from neurones has generally proved to be insensitive to dihydropyridine (Miller & Freedman, 1984). Although transmitter release in general is dihydropyridine-insensitive, high-affinity dihydropyridine binding has been demonstrated on neurones (Miller, 1987). Such observations have produced some confusion and clearly the functions of dihydropyridine-sensitive VSCC in neurones remain to be elucidated. To define further the roles of the various VSCC in neurones, we used brain microdialysis procedure to analyse the effects of an L-type Ca^{2+} channel blocker (nimodipine) and an N-type Ca^{2+} channel antagonist (daurisolone) on transmitter release and Ca^{2+} influx.

Methods

Implantation procedure

Male Sprague-Dawley rats (220–250 g) were used. The animals were placed in a Stoelting stereotaxic apparatus under chloral hydrate (360 mg kg^{-1} , i.p.) anaesthesia. The fibre unit used in the present study was set up as previously described (MacDermott *et al.*, 1986). A single fibre (molecular cut-off 15,000; internal diameter $220 \mu\text{m}$; external diameter $310 \mu\text{m}$) was straightened with a fine wire inserted through its lumen then bent into a loop. Fast-drying glue was spread over the surface of the fibre except on a 4 mm portion at the tip of the loop, to allow exchange with tissue. Before implantation, each probe was flushed through with distilled water for 15 min ($2 \mu\text{L min}^{-1}$) and examined microscopically to ensure that there were no leaks. The completed fibre was then unilaterally implanted into the dorsal hippocampus. The coordinates for implantation were 3.5 mm posterior to bregma, 2.6 mm lateral to the midline, and 2.9 mm below dura. The fibre was fixed to the skull with dental acrylic.

In vitro recovery

Recovery of glutamate and Ca^{2+} from standard solutions. The tips of the dialysis probes were immersed in 2 ml nominally Ca^{2+} -free Krebs-Ringer Bicarbonate (KRB, composition in mM: NaCl 122, KCl 3, MgSO_4 1.2, KH_2PO_4 0.4 and NaHCO_3 25) and placed in a solution of KRB containing CaCl_2 (0.5 mM), glutamate ($10 \mu\text{M}$) at a flow rate of $2 \mu\text{L min}^{-1}$, consecutive six 15 min samples of perfusate were collected. The average recovery of glutamate and Ca^{2+} was determined by use of 3 different fibre units. After 60 min perfusion in KRB + 1 mM CaCl_2 and $100 \mu\text{M}$ glutamate, the fibre was

¹ Author for correspondence.

Figure 1 Time-course changes in the perfusate levels of Ca and glutamate from rat hippocampus perfused unilaterally with veratridine (a), KCl (b) and quinolinic acid (Quin, c). Hippocampus dialysis samples were collected at 15-min intervals, before (open columns, basal level) and after (hatched columns) the perfusion of veratridine (0.1 mM), KCl (75 mM) and Quin (80 mM) through the fibre for 60 min (veratridine, 7 rats) and (KCl, 7 rats) and for 30 min (Quin, 7 rats). The data are presented as mean with s.e.mean shown by vertical bars. A typical example of Ca decrease relative to glutamate changes. The arrows indicate time of the perfusion of stimulants. ** $P < 0.05$, *** $P < 0.01$ vs basal level. Dunnett's two-tailed test.

placed in KRB solution containing 0.5 mM CaCl_2 and 10 μM glutamate for 60 min and then again in KRB + 1 mM CaCl_2 and 100 μM glutamate for another 60 min. This enabled us to check for fibre unit delay in detecting sudden changes in glutamate and Ca^{2+} concentrations of the extracellular compartment.

In vivo perfusion

The conscious rats with the unilaterally implanted fibre, the day after surgery, were simultaneously perfused at a constant rate of 2 $\mu\text{l min}^{-1}$ with KRB, pH 7.4, for measurement of glutamate release, or with KRB nominally Ca^{2+} -free, for determination of Ca^{2+} influx. Fifth min perfusates were collected after 45 min washout. Extracellular Ca^{2+} and glutamate enter the fibre lumen by difference of concentration until equilibrium is reached between two compartments. Changes in the glutamate release and Ca^{2+} influx are then reflected in the Ca^{2+} and glutamate levels of the perfusates. To establish a stable baseline, samples were collected for 60 min before pharmacological treatments; then samples were collected for a minimum of 180 min.

Drug treatment

The drugs were dissolved in vehicles given in parentheses. Collection proceeded for 60 min to determine basal levels of glutamate and Ca^{2+} before the application of drugs in the superfusion stream. After the basal collection period, veratridine (0.1 mM in KRB), quinolinic acid (Quin) (80 mM in KRB) and KCl (75 mM in KRB) were applied through the fibre for 60 min (veratridine, 6–9 rats), 60 min (KCl, 6–8 rats), or 30 min (quinolinic acid, 6–9 rats) to measure the changes of glutamate and Ca^{2+} stimulated by these agents. In the other experiments, specified doses of nimodipine (in propylene glycol) and daurisolone (in propylene glycol) were applied i.v. for 15 min before administration of veratridine, Quin or KCl, to study the direct effects of the drugs on the changes in glutamate and Ca^{2+} induced by these agents.

Glutamate determination

Glutamate was measured by high performance liquid chromatography (h.p.l.c.) after automatic precolumn derivatization with *O*-phthaldialdehyde (OPA) according to the method of Lindroth & Mopper (1979). Thirty μl of the perfusate was mixed with 30 μl of an OPA solution (5.4 mg ml^{-1}) and the reaction product was injected onto a Waters Nucleosil RP-18 h.p.l.c. column 2 min later. The fluorescent reaction products were eluted over a period of 25 min with a mobile phase consisting of 0.1 M citrate: 0.1 M phosphate (4:1) buffer, pH 7.0 and methanol (30–70% at a flow rate of 1 ml min^{-1}). The effluent was monitored fluorimetrically (Waters Fluorescence Detector 420 AC) and the data recorded and computed.

Ca^{2+} measurement

Ca^{2+} was measured in an Automatic Atomic Absorption Spectrophotometer (Model GBC 908 AA): 30 μl of the perfusate was added to 1% HCl (70 μl) and the mixed perfusate injected into the meter, the data recorded and computed.

Histological analysis

Animals were killed after perfusion. The correct position of the fibre in the dorsal hippocampus was verified in every animal during histological assessments. Only animals with the probe in the correct position were used for the statistical analysis.

Statistical analysis

The results were calculated as concentration of glutamate (nmol l^{-1} perfusate) and Ca^{2+} ($\mu\text{mol l}^{-1}$ perfusate). The mean values at each time after drug treatment were compared with the basal level, which was the average of 6–9 samples collected immediately preceding drug application. Significant differences were determined by Dunnett's two-tailed test for multiple comparisons and by the non-parametric Mann-Whitney U-test.

Quin and veratridine were obtained from Sigma Chemical Co (St Louis, MO, U.S.A.). Nimodipine was kindly provided by Tanjing Institute of Pharmaceuticals. Daurisolone was obtained from the Department of Medical Chemistry, China Pharmaceutical University. OPA was purchased from Pierce Chemical Co (Rockford IL, U.S.A.).

Results

In vitro recovery

By dialysis $12 \pm 1.2\%$ of Ca^{2+} and $27.8 \pm 1.9\%$ of glutamate were recovered, expressing the mean concentration in the perfusate as a percentage of the concentration of the solution outside the tube. A decrease or increase in Ca^{2+} and glutamate concentration of the external medium was proportion-

Table 1 Effects of nimodipine and daurisoline on the glutamate release caused by veratridine (0.1 mM) infused into rat hippocampus

Drug (mg kg ⁻¹)	Basal level (nM)	Veratridine (nM)	Stim – Basal (nM)	% inhibition
Nimodipine (0.5)	156.4 ± 13.3 (9)	499.2 ± 47.1 (7)	342.8 ± 21.2	
Nimodipine (0.5)	150.0 ± 13.6 (7)	483.4 ± 44.2 (6)	333.4 ± 19.4	
Daurisoline (0.5)	153.3 ± 12.9 (8)	409.6 ± 39.8 (6)**	256.3 ± 13.1	25
(1)	149.5 ± 11.3 (7)	297.6 ± 23.3 (8)***	148.1 ± 9.9	57
(5)	149.2 ± 11.9 (9)	173.4 ± 15.2 (5)***	24.2 ± 2.3	93

Data represent mean and s.e.mean of glutamate (nM) in each 15-min fraction (100–115 min after beginning of perfusion). Veratridine (0.1 mM) was perfused through the fibre for 60 min. Nimodipine and daurisoline were administered i.v. 15 min before the perfusion of veratridine.

P* < 0.05, *P* < 0.1 vs control alone with Mann-Whitney U-test. The number of animals are given in parentheses.

Stim – Basal was calculated as difference between basal level and veratridine-treated. % inhibition = [1 – (Stim – Basal (Drug))/(Stim – Basal (Control))].

ally reflected in the Ca²⁺ content and glutamate levels of the perfusates in the second 15-min sample collected after changing the solution.

Effects of depolarizing agents and quinolinic acid on control levels of extracellular glutamate in hippocampus dialysates

The basal level of glutamate in hippocampus from the four fractions was 156.8 ± 14.4 nM (mean ± s.e.mean, *n* = 7). The effects of veratridine, KCl and Quin on the extracellular glutamate level are illustrated in Figure 1. When compared with the basal level of glutamate, veratridine and KCl caused a substantial (280–320% of the basal) increase in glutamate level. The glutamate concentration in the perfusate reached a maximum within the 100 min following perfusion of veratridine or KCl and returned to basal over the next 240 min (Figure 1). No significant changes in the glutamate level following perfusion of Quin were detected.

Effects of veratridine, KCl and quinolinic acid on control level of extracellular Ca²⁺ in hippocampus dialysates

A typical example of Ca²⁺ changes relative to glutamate changes is presented in Figure 1. A decrease in Ca²⁺ (40–50% of basal) followed administration of veratridine, KCl and Quin. In particular, glutamate release occurred only following veratridine and KCl but not following Quin. Thus, a clear dissociation between neurotransmitter release and Ca²⁺ influx was demonstrated.

Effects of nimodipine and daurisoline on evoked changes in extracellular glutamate in hippocampus perfusates

Nimodipine was administered i.v. at increasing doses of 0.05, 0.1 and 0.5 mg kg⁻¹ to study the direct effects of the drug on stimulated glutamate release. At all these doses, nimodipine did not produce significant changes in the stimulated glutamate release by depolarizing agents (Tables 1 and 2). In the animal group treated with daurisoline 0.5 mg kg⁻¹, glutamate

Table 2 Effects of nimodipine and daurisoline on the glutamate release caused by KCl (75 mM) infused into rat hippocampus

Drug (mg kg ⁻¹)	Basal level (nM)	KCl (nM)	Stim – Basal (nM)	% inhibition
Control	156.4 ± 13.3 (9)	443.7 ± 39.8 (7)	287.3 ± 26.5	
Nimodipine (0.5)	150.0 ± 13.6 (7)	456.5 ± 41.0 (6)	306.5 ± 32.1	
Daurisoline (0.5)	153.3 ± 12.9 (8)	386.2 ± 32.4 (6)**	232.9 ± 18.7	19
(1)	149.5 ± 11.3 (7)	277.7 ± 20.1 (8)***	128.2 ± 9.8	55
(5)	149.2 ± 11.9 (9)	169.6 ± 14.9 (5)***	20.4 ± 8.1	93

Data represent mean and s.e.mean of glutamate concentration (nM) in each 15-min fraction (100–115 min after beginning of perfusion). KCl (75 mM) was perfused through the fibre for 60 min. Nimodipine and daurisoline were administered i.v. 15 min before the perfusion of KCl.

P* < 0.05, *P* < 0.01 vs control alone, Mann-Whitney U-test. The number of animals are given in parentheses.

Stim – Basal was calculated as difference between basal level and KCl-treated. % inhibition = [1 – (Stim – Basal (Drug))/(Stim – Basal (Control))].

Table 3 Effects of nimodipine and daurisoline on decrease in extracellular Ca²⁺ caused by veratridine (0.1 mM) infused into rat hippocampus

Drug (mg kg ⁻¹)	Basal level (μM)	Veratridine (μM)	Stim – Basal (μM)	% inhibition
Control	91.2 ± 8.7 (9)	48.8 ± 3.9 (7)	–42.4 ± 3.2	
Nimodipine (0.05)	92.0 ± 7.9 (7)	57.8 ± 6.2 (6)**	–34.2 ± 2.9	19
(0.1)	90.9 ± 7.9 (8)	68.1 ± 7.1 (6)**	–22.8 ± 1.4	46
(0.5)	89.0 ± 8.1 (7)	76.5 ± 7.1 (8)***	–12.5 ± 0.9	71
Daurisoline (5)	90.9 ± 9.0 (9)	68.4 ± 6.9 (5)***	–22.5 ± 2.1	47

Data represent mean and s.e.mean of Ca²⁺ concentration (μM) in each 15-min fraction (100–115 min after beginning of perfusion). Veratridine (0.1 mM) was perfused through the fibre for 60 min. Nimodipine and daurisoline were administered i.v., 15 min before the perfusion of veratridine.

P* < 0.05; *P* < 0.01 vs control alone, Mann-Whitney U-test. Number of animals are given in parentheses.

Stim – Basal was calculated as difference between basal level and veratridine-treated. % inhibition = [1 – (Stim – Basal (Drug))/(Stim – Basal (Control))].

release induced by veratridine was significantly reduced (inhibition = 25%, *n* = 6) and KCl-stimulated glutamate release was also blocked (inhibition = 19%, *n* = 6). This effect was even more evident in 5 mg kg⁻¹ reaching an almost complete suppression of glutamate release induced by veratridine and KCl, respectively (inhibition = 93%, *n* = 5).

Effects of nimodipine and daurisoline on the stimulant-induced decrease in extracellular Ca²⁺

Tables 3, 4 and 5 show that Ca²⁺ decreases induced by veratridine, KCl and Quin were antagonized by nimodipine (0.05 mg kg⁻¹). The percentage inhibition was 19 (*n* = 6), 11 (*n* = 6) and 44 (*n* = 6), respectively. A marked blockade of Ca²⁺ decrease by nimodipine (0.5 mg kg⁻¹) was observed (inhibition = 71% (*n* = 7), 71% (*n* = 7), and 92 (*n* = 8), respectively). Veratridine-, KCl- and Quin-stimulated falls in extracellular Ca²⁺ were also prevented by 5 mg kg⁻¹ of daurisoline (inhibition = 47%, *n* = 5; 39%, *n* = 5 and 64%, *n* = 5, respectively). Neither nimodipine nor daurisoline modified the basal Ca²⁺ level.

*

Discussion

In the present study, ‘brain dialysis’ enabled us to measure the extracellular Ca²⁺ changes and transmitter (glutamate) release caused in the hippocampus of a conscious animal by two depolarizing agents (veratridine, KCl) and one excitotoxin (Quin). Our experimental approach does not provide direct evidence of whether Ca²⁺ leaving the extracellular space (reflected in a drop in the ion concentration of the perfusate) enters the intraneuronal compartment. However, most studies have shown that veratridine and KCl-activation raises the cytoplasmic Ca²⁺ concentration in nerve terminals and cell soma (Norris & Bradford, 1985; Mody & Heinemann, 1986) and Ca²⁺ influx stimulated by veratridine and KCl proved to be mediated by VSCC (Nachshen & Blaustein,

1979). Moreover, Ca²⁺ influx by glial cells is not an important site for Ca²⁺ fluxes induced by these depolarizing agents and excitotoxins (Kelly & Krnjevic, 1967; Bowman & Kimelberg, 1984).

A comparative analysis of Ca²⁺ content in brain perfusate and glutamate release at the various times after veratridine or KCl administration showed a close temporal correlation between Ca²⁺ changes and glutamate release. The present data may suggest that an inward Ca²⁺ current is important for triggering glutamate release, although a cause-and-effect relationship cannot be established.

In studies conducted *in vitro*, daurisoline was found to block N-type Ca²⁺ channels selectively (Lu & Liu 1990a, b). In these studies, we had analyzed the depolarization-induced ‘fast’ phase ⁴⁵Ca influx into synaptosomes using the same conditions as had been employed in the [³H]-γ-aminobutyric acid release studies. We observed that ‘fast’ Ca²⁺ influx coupled to neurotransmitter release was potently blocked by daurisoline (IC₅₀ 10⁻⁶ M) but not by nitrendipine. Daurisoline is also less active at L-type channels of smooth muscle cells. More recently, electrophysiological studies (patch clamp) showed that daurisoline inhibited the second inactivating phase of the Ca²⁺ current during strong depolarization in chick dorsal root ganglion (unpublished data). This component of Ca²⁺ current was operated by N-type channels. The ability of daurisoline, which blocked N-type channels, to prevent the fall in Ca²⁺ and glutamate release further suggested that Ca²⁺ influx induced by the agents may be closely involved in the mechanism underlying glutamate release. The inability of Quin, a stimulator of Ca²⁺ influx, to increase glutamate release further suggested that Ca²⁺ influx induced by Qin mainly occurs through VSCC activated by Na⁺ entry in nerve cell soma, not in nerve terminals.

The ability of nimodipine to prevent the fall in Ca²⁺ may be the same mechanisms as for Mg²⁺. Exposure to excess Quin, a potent agonist acting at NMDA receptors, can destroy neurones in the central nervous system and may be responsible for some neurological diseases (i.e. seizures) (Vezzani & Wu, 1988). In this study, we also observed changes

Table 4 Effects of nimodipine and daurisoline on decrease in extracellular Ca²⁺ caused by KCl infused into rat hippocampus

Drug (mg kg ⁻¹)	Basal level (μM)	KCl (μM)	Stim – Basal (μM)	% inhibition
Control	91.2 ± 8.7 (9)	56.4 ± 4.3 (7)	–34.8 ± 2.9	
Nimodipine (0.05)	92.0 ± 7.9 (7)	61.0 ± 6.9 (6)**	–31.0 ± 2.3	11
(0.1)	90.9 ± 7.9 (8)	72.3 ± 7.5 (6)**	–18.6 ± 1.1	47
(0.5)	89.0 ± 8.1 (7)	79.0 ± 8.2 (8)***	–10.0 ± 0.9	71
Daurisoline (5)	90.9 ± 9.0 (9)	69.6 ± 7.1 (5)**	–21.3 ± 1.9	39

Data represent mean and s.e.mean of Ca²⁺ concentration (μM) in each 15-min fraction (100–115 min after beginning of perfusion). KCl (75 mM) was perfused through the fibre for 60 min. Nimodipine and daurisoline were administered i.v. 15 min before the perfusion of KCl. ***P* < 0.05; ****P* < 0.01 vs control alone, Mann-Whitney U-test. Number of animals are given in parentheses. Stim – Basal was calculated as difference between basal level and KCl-treated. % inhibition = [1 – (Stim – Basal (Drug))/Stim – Basal (Control)].

Table 5 Effects of nimodipine and daurisoline on decrease in extracellular Ca²⁺ caused by quinolinic acid (Quin) infused into rat hippocampus

Drug (mg kg ⁻¹)	Basal level (μM)	Quin (μM)	Stim – Basal (μM)	% inhibition
Control	91.2 ± 8.7 (9)	42.2 ± 3.0 (7)	–49.0 ± 3.8	
Nimodipine (0.05)	92.0 ± 7.9 (7)	64.8 ± 5.6 (6)**	–27.2 ± 1.9	44
(0.1)	90.9 ± 7.9 (8)	79.7 ± 8.1 (6)***	–11.2 ± 0.8	77
(0.5)	89.0 ± 8.1 (7)	85.0 ± 8.6 (8)***	–4.0 ± 0.2	92
Daurisoline (5)	90.9 ± 9.0 (9)	73.4 ± 7.4 (5)***	–17.5 ± 1.1	64

Data represent mean and s.e.mean of Ca²⁺ concentration (μM) in each 15-min fraction (100–115 min after beginning of perfusion). Quin (80 mM) was perfused through the fibre for 30 min. Nimodipine and daurisoline were administered i.v., 15 min before the perfusion of Quin. ***P* < 0.05, ****P* < 0.01 vs control alone, Mann-Whitney U-test. Number of animals are given in parentheses. Stim – Basal was calculated as difference between basal level and Quin-treated. % inhibition = [1 – (Stim – Basal (Drug))/Stim – Basal (Control)].

in animal behaviour induced by Quin. The animals presented episodes of tonic rotation of the head, associated with chewing movements. This abnormal animal behaviour was completely blocked by nimodipine (0.5 mg kg^{-1}) but not by daurisoline. In fact, we recently reported an antiepileptic effect of nimodipine on excitatory amino acid-induced seizures. The mechanisms involved blocking Ca^{2+} influx into neurones (Lu *et al.*, 1990). This may form the basis of seizures induced by Quin. Therefore, Ca^{2+} channels in cell soma rather than in nerve terminals were responsible for this disorder, because nimodipine can inhibit Quin-induced seizures (Morocutt *et al.*, 1986; Lu *et al.*, 1990). In these studies, no correlations were found between blocking Ca^{2+} influx and seizures induced by Quin, even with large doses of daurisoline. The explanation for this result is that the inhibition of Ca^{2+} influx by daurisoline here, as a consequence of antagonizing Quin-stimulated protein kinase C (CPK) (Lu & Zhao, 1991), did not directly block the VSCC in soma. This did not sufficiently block the excess Ca^{2+} influx evoked and did not prevent convulsions.

In contrast to the effects of daurisoline on glutamate release, the glutamate release evoked by depolarizing agents has been shown to be insensitive to nimodipine, yet Ca^{2+} influx was blocked by nimodipine. How can these observations be explained? It is clear that stimulus-secretion coupling is predominantly mediated by dihydropyridine-insensitive N-type VSCC. Nowicky *et al.* (1985a, b) recently advanced our understanding significantly by demonstrating that chick dorsal root ganglion (DRG) neurones in culture possessed

three distinct types of VSCC. The second type of VSCC (N), of intermediate size, was responsible for the second inactivating phase of the Ca^{2+} current, observed during strong depolarizations. The third VSCC (L), which was also activated by strong depolarizations, was responsible for the non-inactivating component of the current. The pharmacological properties of these types of VSCC proved most interesting. L-type channels were modulated by dihydropyridine agonists and antagonists such as nimodipine, but N-channels were not. Rapid ^{45}Ca flux measurements in synaptosomes have shown that the VSCC that mediate transmitter release in most cases also exhibit voltage inactivation and are nimodipine-insensitive (Lu & Liu, 1990a; Nachshen & Blaustein, 1979). They closely resemble the N-type channels described electrophysiologically. In most cases, such channels may form clusters around transmitter release sites on nerve terminals. It is quite likely that L-type channels may not be very common in nerve terminals and may be preferentially localized in the cell soma, since the concentration of [^3H]-nimodipine receptors is much higher in the postsynaptic membrane than on the presynaptic terminal (Pumplin *et al.*, 1991). Our results provide further evidence for this hypothesis. *

Our results show that glutamate release and Ca^{2+} influx may be dissociated by different stimulants and differently blocked by calcium antagonists. Daurisoline, by potently inhibiting Ca^{2+} influx and glutamate release, appears to be a useful tool to study VSCC.

References

- BOWMAN, C.L. & KIMELBERG, H.K. (1984). Excitatory amino acids directly depolarize rat brain astrocytes in primary culture. *Nature*, **311**, 656–659.
- CARBONE, E. & LUX, H.D. (1984). A low voltage activated, fully inactivating Ca channel in vertebrate sensory neurones. *Nature*, **310**, 501–502.
- FREEDMAN, S.B. & MILLER, R.J. (1984). Calcium channel activation: A different type of drug action. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 5580–5583.
- KELLY, J.S., KRNEVIC, K. & YIM, G.K. (1967). Unresponsive cells in cerebral cortex. *Brain Res.*, **6**, 767–769.
- LINDROTH, P. & MOPPER, K. (1979). High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with *O*-phthalaldehyde. *Anal. Chem.*, **51**, 1667–1674.
- LU, Y.M. & LIU, G.Q. (1990a). Effects of (–)-daurisoline on Ca influx into presynaptic nerve terminals. *Br. J. Pharmacol.*, **101**, 45–48.
- LU, Y.M. & LIU, G.Q. (1990b). Effects of (–)-daurisoline on quinolinic acid-induced Ca influx in neurons in freely moving rats. *Acta Pharmacol. Sin.*, **12**, 301–304.
- LU, Y.M., ZHANG, J.T. & ZHAO, F.Q. (1990). Effects of nimodipine on L-glutamate induced seizures and Ca influx in the hippocampus in freely moving rats. *Acta Pharmacol. Sin.*, **12**, 297–300.
- LU, Y.M. & ZHAO, F.Q. (1991). Effects of (–)-daurisoline on CPK-activities in synaptosomes. *Chin Pharmacol. Bull.*, **7**, 174–179.
- MILLER, R.J. (1987). Multiple calcium channels and neuronal function. *Science*, **235**, 46–52.
- MILLER, R.J. & FREEDMAN, S.B. (1984). Minireview: are dihydropyridine binding sites voltage-sensitive calcium channels? *Life Sci.*, **34**, 1205–1221.
- MACDERMOTT, A.B., MAYER, M.L., WESTBROOK, G.L., SMITH, S.J. & BARKER, J.L. (1986). NMDA-receptor activation increase cytoplasmic calcium concentration in culture spinal cord neurones. *Nature*, **321**, 519–522.
- MODY, T. & HEINEMANN, U. (1986). Laminar profiles of the changes in extracellular calcium concentration induced by repetitive stimulation and excitatory amino acids in the rat dentate gyrus. *Neurosci. Lett.*, **69**, 137–142.
- MOROCUTT, C., PIERELLI, F. & SANARELLI, L. (1986). Antiepileptic effects of a calcium antagonist (nimodipine) on cefazoline-induced epileptogenic foci in rabbits. *Epilepsia*, **27**, 498–503.
- NACHSHEN, D.A. & BLAUSTEIN, M.P. (1979). The effects of some 'organic calcium antagonists' on calcium influx in presynaptic nerve terminals. *Mol. Pharmacol.*, **16**, 579–586.
- NORRIS, D.K. & BRADFORD, H.F. (1985). On the specificity of verapamil as a calcium channel-blocker. *Biochem. Pharmacol.*, **34**, 1953–1956.
- NOWICKY, M.C., FOX, A.P. & TSIEN, R.W. (1985a). Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature*, **316**, 440–443.
- NOWICKY, M.C., FOX, A.P. & TSIEN, R. W. (1985b). Long-opening mode of gating of neuronal calcium channels and its promotion by its dihydropyridine calcium agonist Bay K 8644. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 2178–2182.
- PUMPLIN, D.W., REESE, T.S. & LLINAS, R. (1981). Are the presynaptic membrane particles the calcium channels? *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 7210–7216.
- TRIGGLE, D.J. & RAMPE, D. (1989). 1,4-dihydropyridine activators and antagonists: structural and functional distinctions. *Trends Pharmacol. Sci.*, **10**, 507–511.
- VEZZANI, A. & WU, H.Q. (1988). 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.*, **454**, 289–297.

(Received July 11, 1990
Revised April 29, 1991
Accepted May 14, 1991)