Journal of Neurochemistry
Raven Press, Ltd., New York
© 1992 International Society for Neurochemistry

N-Methyl-D-Aspartate–Mediated Injury Enhances Quisqualic Acid–Stimulated Phosphoinositide Turnover in Perinatal Rats

Chu-Kuang Chen, *†Faye S. Silverstein, and ‡Michael V. Johnston

*Neuroscience Program, and Departments of *Pediatrics and †Neurology, University of Michigan, Ann Arbor, Michigan, and ‡The Kennedy Research Institute and Johns Hopkins University Medical Institutions, Baltimore, Maryland, U.S.A.*

Abstract: Previous work in our laboratory demonstrated that ischemic–hypoxic brain injury in postnatal day 7 rats causes a substantial increase in phosphoinositide (PPI) turnover stimulated by the glutamate analogue quisqualic acid (QUIS) in the hippocampus and striatum. To examine this phenomenon in more detail, we performed similar experiments after producing injury by unilateral intracerebral injections of the glutamate analogue *N*-methyl-D-aspartate (NMDA). The 7-day-old rodent brain is hypersensitive to NMDA neurotoxicity and NMDA injection causes histopathology that closely resembles that produced by ischemia–hypoxia. NMDA, 17 nmol in 0.5 μl, was injected into the right posterior striatum of 7-day-old rat pups and they were killed 3 days later. Hippocampal or striatal tissue slices were prepared from ipsilateral and contralateral hemispheres from vehicle-injected control and from noninjected control rat pups. Slices were then incubated with *myo*-[^{3}H]inositol plus glutamate agonists or antagonists in the presence of lithium ions and [^{3}H]inositol monophosphate ([^{3}H]IP$_1$) accumulation was measured. The glutamate agonists, QUIS, L-glutamic acid, and (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, stimulated greater [^{3}H]IP$_1$ release in tissue ipsilateral to the NMDA injection compared with that in the contralateral side and in control pups. The glutamate antagonists, D,L-2-amino-7-phosphonoheptanoic acid, 3-[(+)-2-carboxypiperazin-4-yl]-propyl-1-phosphoric acid, kynurenic acid, and 6,7-dinitroquinoxaline-2,3-dione did not inhibit QUIS-stimulated [^{3}H]IP$_1$ release. The enhanced PPI turnover in the lesioned tissue was specific to glutamate receptors because carbachol (CARB) failed to elicit preferential enhanced stimulation. To investigate the possibility that alterations in the release of endogenous neurotransmitters had a role in potentiating QUIS-stimulated PPI turnover after NMDA injection, we examined the effect of tetrodotoxin. Tetrodotoxin (0.5 μ*M*) did not alter QUIS- or CARB-stimulated PPI hydrolysis in the lesioned or unlesioned tissue. The influence of extracellular calcium concentration on QUIS-stimulated [^{3}H]IP$_1$ formation was also examined after the NMDA lesion. Moderate reduction of calcium in the buffer (1 μ*M*) enhanced the lesion effect. Low calcium buffer enhanced QUIS-stimulated PPI turnover in the lesioned hippocampal slices, but reduced QUIS stimulation in contralateral slices and controls. In contrast, CARB-stimulated PPI turnover was not enhanced in low Ca^{2+} buffer. A similar pattern of Ca^{2+} dependency was observed in striatal slices. Calcium-free (<10 n*M*) buffer suppressed PPI turnover in all groups. These studies demonstrate that NMDA-induced excitotoxic injury in neonatal rats causes a selective enhancement of QUIS-stimulated PPI turnover that resembles the effects of ischemia–hypoxia. In addition, we found that agonist-stimulated PPI turnover is sensitive to the in vitro Ca^{2+} concentration. These changes could reflect altered coupling of non-NMDA receptors to phospholipase C activity. **Key Words:** Excitotoxicity—Inositol phosphate—Immature brain—Calcium. **Chen C.-K. et al.** *N*-Methyl-D-aspartate–mediated injury enhances quisqualic acid–stimulated phosphoinositide turnover in perinatal rats. *J. Neurochem.* **59,** 963–971 (1992).

In the developing nervous system, activation of a subpopulation of excitatory amino acid (EAA) receptors is coupled to activation of phospholipase C (PLC). PLC catalyzes the hydrolysis of membrane inositol phospholipids (PPIs), especially phosphatidylinositol 4,5-bisphosphate, and yields two intracellular second messenger molecules, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. IP_3 liberates Ca^{2+} from

Received November 12, 1991; revised manuscript received February 6, 1992; accepted March 2, 1992.

Address correspondence and reprint requests to Dr. M. V. Johnston at Kennedy Research Institute, Johns Hopkins University, 707 N. Broadway, Baltimore, MD 21205, U.S.A.

The present address of Dr. C.-K. Chen is Kennedy Research Institute, 707 N. Broadway, Baltimore, MD 21205, U.S.A.

Abbreviations used: AMPA, (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ANOVA, analysis of variance; AP4, D,L-2-amino-4-phosphonobutyric acid; AP7, D,L-2-amino-7-phosphonoheptanoic acid; CARB, carbachol; CPP, 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphoric acid; DNQX, 6,7-dinitroquinoxaline-2,3-dione; EAA, excitatory amino acid; Glu, L-glutamic acid; G protein, GTP-binding protein; IP_3, inositol 1,4,5-trisphosphate; MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]-cyclohepten-5,10-imine hydrogen maleate; NMDA, *N*-methyl-D-aspartate; PLC, phospholipase C; PPI, inositol phospholipid; QUIS, quisqualic acid; TTX, tetrodotoxin.

intracellular sites and modulates the intracellular calcium level. Compared with other glutamate analogues, quisqualic acid (QUIS), a non-*N*-methyl-D-aspartate (NMDA) glutamate receptor agonist, is one of the most potent activators of PLC in neonatal rat brain. QUIS-stimulated PPI hydrolysis is 10-fold greater in the neonatal hippocampus and striatum than in the adult (Nicoletti et al., 1986*a*).

Injury appears to enhance the ability of EAAs to stimulate PLC activity in both mature and immature brains. We demonstrated that ischemic–hypoxic brain injury in 7-day-old rats enhances QUIS-stimulated PPI turnover in the injured hippocampus and striatum, whereas cholinergic receptor coupled PPI turnover is not changed (Chen et al., 1988). In adult rats, a similar pattern of enhanced ibotenate-stimulated PPI turnover is detected in striatum lesioned with kainic acid (Nicoletti et al., 1987). The mechanism for this selective enhancement of EAA-stimulated PLC activity by injury is unknown. In this study, we examined this phenomenon by studying another type of injury in the developing brain mediated by the EAA analogue NMDA. Intrastriatal injection of NMDA into perinatal rats produces a brain lesion that has a pathology similar to that observed after ischemic–hypoxic brain injury (McDonald et al., 1988; Ikonomidou et al., 1989) and NMDA neurotoxicity is enhanced on postnatal day 7 compared with the adult. The lesion may reproduce the overstimulation of EAA receptors that is thought to play a role in ischemic–hypoxic injury.

In these experiments, lesions were produced by NMDA microinjection in 7-day-old rat pups, and the pups were killed 1–33 days later. In slices prepared from these animals, QUIS-stimulated formation of [^{3}H]IP$_1$ was assayed in the presence of Li^+. Experiments were aimed at quantifying the degree to which PPI turnover was enhanced after injury, the time course of the effect, and the biochemical receptor mechanisms responsible for the enhancement.

MATERIALS AND METHODS

Materials

myo-[2-^{3}H]Inositol (15 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO, U.S.A.). Dowex-1 X8 ion-exchange resin (100–200 mesh; formate form) was obtained from Bio-Rad (Richmond, CA, U.S.A.). QUIS was obtained from Cambridge Research Biochemicals (Cambridge, U.K.). MK-801 [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine hydrogen maleate] was a gift of Dr. P. Anderson from Merck Sharp and Dohme (West Point, PA, U.S.A.). (*RS*)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), D,L-2-amino-7-phosphonoheptanoic acid (AP7), 3-[(±)-2-carboxypiperazin-4-yl]-propyl-1-phosphoric acid (CPP), and 6,7-dinitroquinoxaline-2,3-dione (DNQX) were purchased from Tocris Neuramin (Essex, U.K.). Kynurenic acid, carbachol (CARB; carbamoylcholine), NMDA, D,L-2-amino-4-phosphonobutyric acid (AP4), and other chemicals were obtained from Sigma Chemical (St. Louis, MO, U.S.A.).

Intracerebral NMDA lesions and sham injections

In three assay runs, we compared [^{3}H]IP$_1$ accumulation in vehicle-injected and untreated littermate control rat pups. In 7-day-old Sprague–Dawley rat pups, maintained under ether anesthesia, HEPES (0.5 *M*, pH 7.4) was stereotaxically injected into the posterior striatum by Hamilton syringe over 1.5 min. The stereotaxic coordinates were AP = +2, L = 2.5, D = +4.0 in relation to bregma. These vehicle-injected rats and untreated littermates were killed 3 days later. Samples of tissue from 10 rats were pooled for each group and were assayed in triplicate. For NMDA lesion studies, NMDA (17 nmol, dissolved in 0.5 μl of HEPES buffer, pH 7.4) was stereotaxically injected into the posterior striatum. In most experiments, NMDA-injected (18–24 rats/assay pooled) rats and untreated littermate control rats (12 rat pups/assay pooled) were killed 3 days later. Increased animal numbers in lesioned groups were used to provide enough tissue to assay the pooled sample in triplicate across all parameters. For some experiments, additional groups of rats were killed 1, 6, and 33 days after NMDA injection.

To examine whether an NMDA lesion in the left hemisphere affected receptor-coupled PPI hydrolysis to the same degree as right-sided injury, in one assay run (16 rat pups pooled), we injected NMDA (17 nmol) into the left striatum of 7-day-old rat pups with the same coordinates as described above but left of bregma. Three days later, hippocampal and striatal tissue was collected and assayed with untreated controls in the same way as described above.

Histopathology was assessed in Nissl-stained coronal brain sections (two animals each) at days 1, 3, 6, and 33 after intrastriatal injection of 17 nmol of NMDA. Histology of vehicle-injected rats was also done.

Tissue preparation and assay of PPI metabolism

The hippocampus and striatum were dissected out and cross-chopped brain slices (350 × 350 μm) were prepared with a McIlwain chopper. For each anatomic region, this tissue was used to prepare three pooled samples from the side of injection, the contralateral hemisphere, and control pups. Slices were suspended and washed three times with warm (37°C) buffer (142 m*M* NaCl, 5.6 m*M* KCl, 2.2 m*M* $CaCl_2$, 3.6 m*M* $NaHCO_3$, 1 m*M* $MgCl_2$, 5 m*M* D-glucose, and 30 m*M* Na^+-HEPES, pH 7.4). After the slices settled by gravity, excess buffer was aspirated and a 20-μl aliquot of tissue (protein content, 0.1–0.4 mg) was transferred to test tubes containing 4.0 μCi of *myo*-[2-^{3}H]inositol, LiCl (10 m*M*), and specific agonists or antagonists in a final volume of 500 μl of buffer. This mixture was then incubated at 37°C for 2 h. This continuous labeling paradigm was used because of its simplicity and sensitivity (Berridge et al., 1982; Fisher and Bartus, 1985; Heacock et al., 1987).

Two hours after incubation, reactions were terminated by adding 1.7 ml of chloroform/methanol (1:2 by volume) and then left overnight. [^{3}H]IP$_1$ accumulation was monitored as described previously (Berridge, 1982; Fisher and Bartus, 1985). In brief, 1 ml of chloroform and 0.5 ml of water were added and the water-soluble and organic phases were separated by centrifugation. The aqueous samples were removed, diluted with 1.5 ml of water, and then applied to 0.5-ml columns of AG 1X-8 resin (formate form) equilibrated with distilled water. The columns were first washed with 42 ml of 5 m*M* sodium tetraborate/60 m*M* sodium formate to elute glycerophosphorylinositol. The inositol monophosphate fraction was then eluted with 18 ml of 0.1 *M* formic acid/0.2 *M* ammonium formate solution, and

radioactivity determined. To monitor the incorporation of labeled *myo*-inositol into the phospholipid fraction, a 0.2-ml aliquot from the organic phase was removed and radioactivity was measured (Berridge et al., 1982; Fisher and Bartus, 1985).

Three groups of experiments were performed for both injured and uninjured hippocampal and striatal tissue. First, we examined the effects of glutamate agonists, antagonists, and other neurotransmitter agonists on PPI turnover. Then, we examined the effect of tetrodotoxin (TTX) on QUIS-stimulated PPI turnover. Finally, we determined how the calcium content of the incubation buffer modulated the extent of QUIS-stimulated PPI hydrolysis.

Pharmacologic studies of QUIS-stimulated PPI hydrolysis after NMDA lesions

Rats were killed at four time points after NMDA injection on postnatal day 7 (1 day after, two assay runs; 3 days, nine assays; 6 days, one assay; 33 days, two assays; 16–24 pups/assay). Hippocampal and striatal tissue slices were prepared from tissue of the injected side, noninjected side, and control pups, and incubated with glutamate agonist QUIS (10^{-5} *M*) as described above. Dose–response studies were performed on hippocampal tissue prepared 3 days after NMDA injection. These tissue slices were incubated with 10^{-9} to 10^{-3} *M* QUIS for 2 h and the [^{3}H]IP$_1$ formation was monitored (four assays, 16–24 pups per assay pooled).

We tested five glutamate agonists [QUIS, L-glutamic acid (Glu), NMDA, AMPA, and AP4], three NMDA antagonists (AP7, CPP, and MK-801), and two non-NMDA antagonists (kynurenic acid and DNQX) to pharmacologically characterize QUIS-stimulated PPI hydrolysis. CARB, a muscarinic agonist that stimulates PPI hydrolysis, was also examined concurrently and used as a comparison.

We investigated whether QUIS and CARB receptors are located on the same cell population by examining [^{3}H]IP$_1$ formation elicited by the combined addition of QUIS and CARB for both hippocampal and striatal tissue slices prepared from the unlesioned control pups.

In an experiment to block endogenous neurotransmitter release, TTX was dissolved in HEPES (50 m*M*, pH 7.4) solution and added at the beginning of the QUIS incubation period (final concentration of TTX, 0.5 μM).

Incubation of samples in Ca(+), Ca(−), and Ca(−) + EGTA buffers

In five assays, we examined the influence of changes in the concentration of Ca^{2+} in the incubation buffer on QUIS-stimulated PPI hydrolysis. Tissue aliquots were incubated with regular buffer containing $CaCl_2$: Ca(+); Ca(−) buffer in which $CaCl_2$ was replaced by water; or Ca^{2+}-free buffer containing 0.5 m*M* EGTA: Ca(−) + EGTA. The calcium concentrations in these three buffers were 2.2 m*M*, 1 μM, and <10 n*M*, respectively, as measured by using Fluo-3 fluorescence measurement (courtesy of Dr. S. K. Fisher). Tissue slices were washed three times with warm (37°C) Ca(−) buffer, and aliquots were then distributed into tubes containing each of these buffers and incubated with agonist as described above.

Data analysis

Data were normalized by protein content in each sample tissue and expressed as disintegrations per minute per milligram protein. Protein concentration was determined according to Lowry et al. (1951). Experiments (below) established that there was no difference between vehicle-injected controls, the contralateral side of NMDA-injected animals, and uninjected control pups. Paired and Student's *t* test and analysis of variance (ANOVA) were used for data analysis. Estimation of EC_{50} for QUIS-stimulated PPI turnover was performed using a pharmacologic calculation computer program, PHARM/PCS (Microcomputer Specialist, PA, U.S.A.).

RESULTS

Histologic changes after NMDA injection

In rats killed 1 day after injection, there was both gross and microscopic evidence of tissue injury in the NMDA-injected hemisphere. Nissl-stained sections showed loss of staining in striatum, hippocampus, thalamus, and overlying cortex limited to the lesioned hemisphere. In hippocampus, all of the CA1, CA2, CA3, and dentate were involved. In animals killed 3 days after injection, the lesioned hemisphere was pale and edematous, whereas the contralateral hemisphere did not show any gross damage. Coronal brain sections showed that the injury and tissue loss occurred in the striatum, hippocampus, and cortex ipsilateral to the side of NMDA injection (Fig. 1). Six days after injection, the degree of edema decreased. Microscopically, the loss of Nissl staining was greater than 3 days after injection. Thirty-three days after injection, the injected hemisphere appeared atrophied and there was a great loss (~80%) of tissue in the hippocampus, striatum, and surrounding cortex. In vehicle-injected animals, there was no gross damage in the hemisphere ipsilateral to injection.

Effect of vehicle injection on PPI turnover

In a pilot study, PPI turnover was measured in vehicle-injected and uninjected littermate rats (three assays, 10 pups/assay for vehicle-injected or uninjected group) to determine if the vehicle itself enhanced PPI turnover. QUIS- and CARB-stimulated and basal PPI hydrolysis in hippocampal and striatal slices was measured 3 days later. There was no significant difference in [^{3}H]IP$_1$ formation between tissue ipsilateral and contralateral to the vehicle injection and untreated animals in hippocampus or striatum (by ANOVA, data not shown).

Because there was no difference between brain tissue from animals injected with vehicle and uninjected animals, tissue from uninjected littermate animals served as a control for NMDA-injected animals. Hippocampal and striatal tissue slices were prepared from tissue of the NMDA-injected side, the uninjected side, and untreated littermate control pups.

QUIS-stimulated PPI hydrolysis is enhanced after NMDA injection

Rats were killed at four time points after NMDA lesioning on postnatal day 7 (1 day after, two assays; 3 days, nine assays; 6 days, one assay; 33 days, two assays; 16–24 pups/assay run). At each time point,

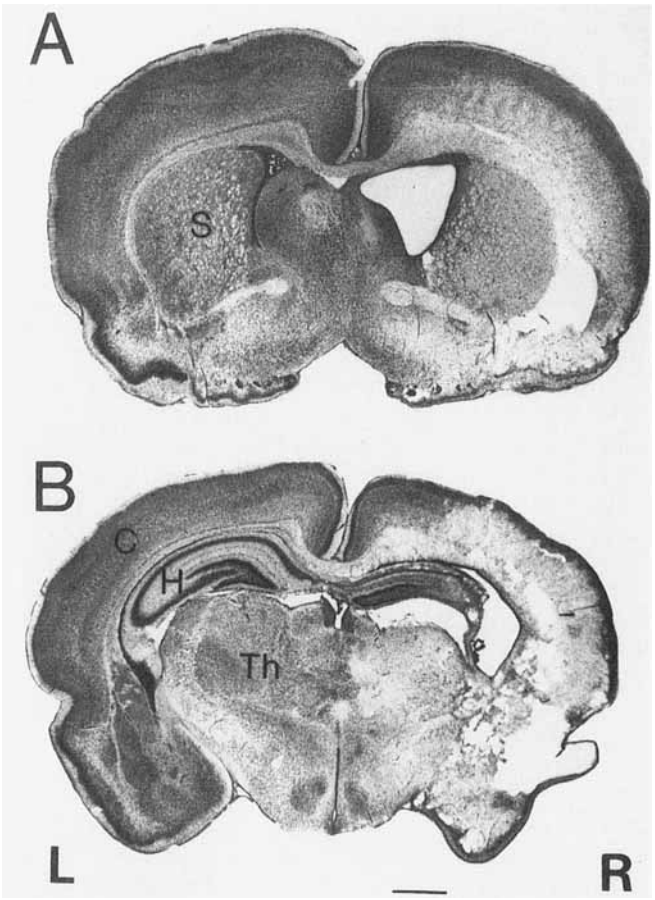

FIG. 1. Photographs of Nissl-stained brain sections from 10-day-old rats at striatal (**A**) and hippocampal (**B**) level. The animals received a unilateral intrastriatal NMDA (17 nmol) injection 3 days earlier (postnatal day 7). Extensive loss of substance is apparent in ipsilateral striatum, hippocampus, and overlying cortex. S, striatum; C, cortex; H, hippocampus; Th, thalamus; L, left side; R, right side. Bar = 1 mm.

QUIS-stimulated PPI hydrolysis in the lesioned hippocampal tissue was considerably higher than that in the unlesioned tissue (Fig. 2). This enhanced QUIS-stimulated PPI breakdown was maximal 1 day after injection (2,000% of baseline), declining subsequently thereafter. Basal (unstimulated) $[^3H]IP_1$ formation was also compared in these tissue slices. On postinjection day 1, basal $[^3H]IP_1$ release was higher in the lesioned than in the contralateral group ($p < 0.05$). There was no difference in basal $[^3H]IP_1$ formation between lesioned and unlesioned hippocampal slices at later time points. Also, there were no differences in QUIS-stimulated and basal PPI hydrolysis between unlesioned and control pups at all time points (data not shown). In striatal slices, the time courses of QUIS-stimulated and basal PPI hydrolysis were similar to that observed for hippocampal slices (data not shown). To facilitate the analysis of agonist-stimulated $[^3H]IP_1$ release, we used animals killed 3 days after NMDA injection for subsequent experiments.

Three days after intrastriatal NMDA was administered by injection, QUIS (10^{-5} *M*) stimulation of $[^3H]IP_1$ release was greater in the lesioned ipsilateral hippocampal (by 123%) and striatal tissue than in the tissue from the contralateral side (by 156%) (Fig. 3). In one assay, we found that there was no difference in the effect when NMDA was injected into the opposite (left) side of the brain and the remainder of the experiments were performed with right-sided lesions.

CARB-stimulated PPI hydrolysis was unchanged after NMDA injection

In contrast to QUIS stimulation, CARB (10^{-2} *M*)-stimulated $[^3H]IP_1$ release was the same in hippocampal (seven assays) and striatal (eight assays) tissue from both hemispheres.

To address the issue of whether QUIS and CARB receptors were present on the same cell population, we determined the stimulation of $[^3H]IP_1$ release elicited by addition of either QUIS (10^{-5} *M*) or CARB (10^{-2} *M*) alone, or when combined in unlesioned tissue. The combination of both QUIS and CARB resulted in less than additive $[^3H]IP_1$. Thus, in 10-day-old rat hippocampus (n = 4 assays), the values for $[^3H]IP_1$ release (dpm/mg of protein) elicited by the addition of QUIS or CARB alone were 52,080 ± 1,680 and 30,900 ± 1,500, respectively. In the presence of both agonists, $[^3H]IP_1$ formation was 57,250 ± 3,400. In striatum (n = 2 assays), the comparable values were 35,260 ± 1,150, 27,750 ± 3,300, and 48,030 ± 3,550.

Dose responses for QUIS-stimulated $[^3H]IP_1$ release

Dose–response curves for QUIS-stimulated PPI turnover were constructed using tissue obtained ipsilaterally or contralaterally to the site of lesion (Fig. 4).

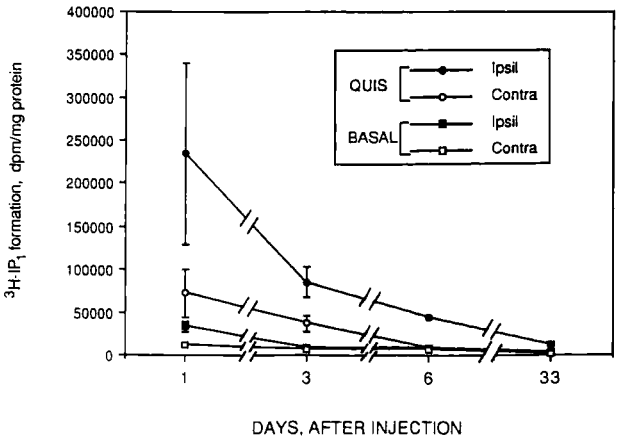

FIG. 2. Stimulation of PPI hydrolysis by QUIS in hippocampal slices at four times after intrastriatal NMDA (17 nmol) injection on postnatal day 7. Slices were prepared from hippocampus ipsilateral (Ipsil) and contralateral (Contra) to NMDA injection and incubated with QUIS (10^{-5} *M*, open and closed circles) or buffer (BASAL; open and closed squares). Values (dpm/mg of protein) are expressed as means ± SEM.

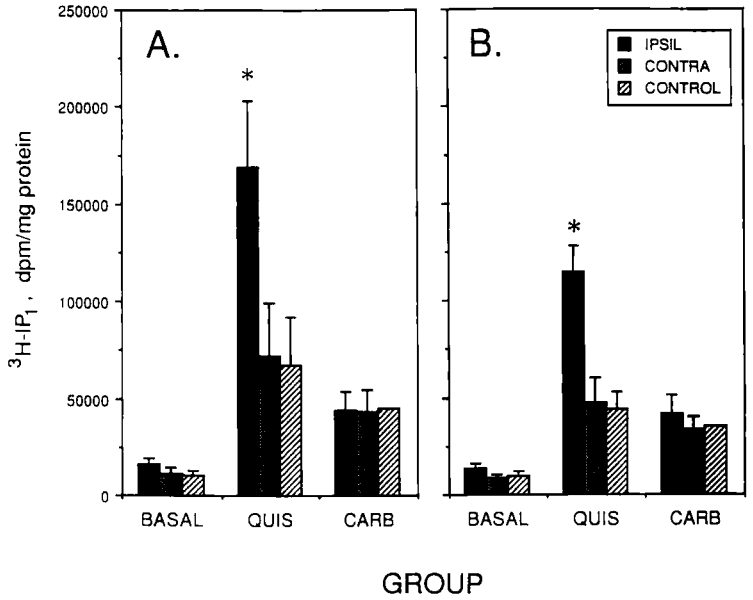

FIG. 3. A: QUIS and CARB-stimulated [^{3}H]IP$_1$ formation in hippocampal tissue 3 days after unilateral intrastriatal injection of 17 nmol of NMDA on postnatal day 7. Tissue slices were prepared from hippocampus ipsilateral (IPSIL) and contralateral to the injection (CONTRA), and from untreated controls (CONTROL). Tissue slices were incubated with *myo*-[2-^{3}H]-inositol in the presence of QUIS (10^{-5} *M*) or CARB (10^{-2} *M*) and buffers (BASAL) (see Materials and Methods for details of assay). Values are [^{3}H]IP$_1$ formation (dpm/mg of protein) and expressed as the means ± SEM. These data are triplicate results from 7 (QUIS) and 8 (CARB) assays, respectively. CARB stimulation in CONTROL slices was only assayed once. **B:** Corresponding values for QUIS- and CARB-stimulated [^{3}H]IP$_1$ release in striatum assayed concurrently. *$p < 0.05$, comparison of values from ipsilateral and contralateral hemispheres (post-hoc test, Fisher's PLSD method, after ANOVA).

Maximal [^{3}H]IP$_1$ release (1,379 ± 114%) of unstimulated littermate control pups was obtained at 10^{-5} *M* QUIS. Higher concentrations of QUIS (10^{-4} and 10^{-3} *M*) showed a decreased stimulation. The EC_{50} values were calculated from the stimulation of PPI turnover at concentrations between 10^{-9} and 10^{-5} *M*. These values were the same in ipsilateral and contralateral hippocampus (1.2×10^{-7} vs. 6.0×10^{-8} *M*) and striatum (1.1×10^{-7} vs. 5.7×10^{-8} *M*). EC_{50} estimates for QUIS-stimulated PPI turnover in control samples were similar (hippocampus, 4.6×10^{-8} *M*; striatum, 6.7×10^{-8} *M*).

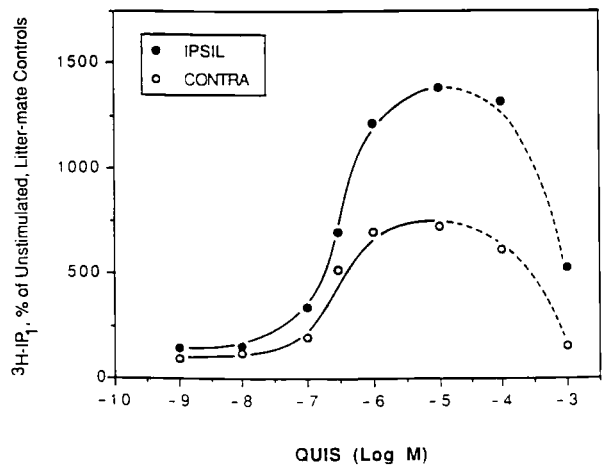

FIG. 4. Dose-dependent stimulation of PPI hydrolysis by QUIS in rat hippocampal slices from tissue ipsilateral (filled circle) or contralateral (open circle) to the NMDA injection side 3 days after injection. Values are the percentage of unstimulated (basal) [^{3}H]IP$_1$ formation in the untreated control rat pups. Data presented here are the mean of the triplicate results from one representative experiment repeated four times with similar results. SEM is within 10% of the mean and not shown in this figure. The curve through each data point was fitted by eye. Values observed at 10^{-9} to 10^{-5} *M* were used to calculate EC_{50} values (see Results).

Effects of EAA agonists and antagonists

Several glutamate agonists and antagonists were tested for their abilities to modify the stimulation of [^{3}H]IP$_1$ release elicited by the addition of QUIS (see Table 1). There was no difference in the extent of QUIS-stimulated PPI hydrolysis between the tissue contralateral to NMDA injection and noninjected control pups; only values from the NMDA-lesioned and unlesioned tissue were therefore compared. NMDA antagonists, AP7 (10^{-3} *M*, two assays) CPP (10^{-3} *M*, two assays), and non-NMDA antagonists, DNQX (10^{-4} *M*, two assays) and kynurenic acid (10^{-3} *M*, one assay) did not stimulate [^{3}H]IP$_1$ release (data not shown). QUIS-stimulated PPI turnover was not affected by competitive NMDA antagonists AP7 and CPP and non-NMDA antagonists kynurenate and DNQX (data not shown). Only high concentrations of noncompetitive NMDA antagonist MK-801 (10^{-3} *M*) inhibited QUIS-stimulated PPI hydrolysis nonselectively in tissue from ipsilateral (by 80%) and contralateral (by 90%) hemispheres (see Table 1). Lower concentrations of MK-801 (<100 μM) failed to inhibit the QUIS stimulation of [^{3}H]IP$_1$ release (data not shown).

Among glutamate agonists, glutamic acid (n = 1 assay, data not shown) and AMPA also elicited this preferential stimulation in the lesioned tissue. In one assay, NMDA (10^{-3} *M*) did not elicit this preferential stimulation in the lesioned tissue despite that it mildly stimulated [^{3}H]IP$_1$ release (40% increase) in both lesioned and unlesioned hippocampal tissue; however, in the same assay, NMDA failed to stimulate PPI hydrolysis in both lesioned and unlesioned striatal tis-

TABLE 1. *Effects of QUIS and related agonists and antagonists of [^{3}H]IP$_1$ accumulation*

		Hippocampus		Striatum	
		Contralateral	Ipsilateral	Contralateral	Ipsilateral
Basal	9	6,980 ± 440	9,170 ± 1,060[a]	8,670 ± 2,240	9,890 ± 2,960
MK-801 (10^{-3} *M*)	3	4,410 ± 1,560	8,690 ± 520	6,080 ± 2,940	18,300 ± 4,590
QUIS (10^{-4} *M*)	4	28,510 ± 17,680	78,980 ± 29,270[a,b]	25,460 ± 10,460[c]	92,340 ± 23,070[a,b]
QUIS (10^{-5} *M*)	9	36,960 ± 9,310[b]	85,820 ± 18,450[b,d]	30,990 ± 6,480[c]	79,180 ± 11,310[c,d]
+ MK-801	3	4,140 ± 2,830[f]	16,120 ± 5,380[f]	3,320 ± 490[f]	30,140 ± 4,450[f]
AMPA (10^{-3} *M*)	5	13,550 ± 2,540	21,970 ± 2,500[b,e]	15,090 ± 1,980[c]	32,330 ± 3,760[b,e]
+ MK-801	1	5,350	17,720	2,140	17,000

Tissue was prepared from the rats killed 3 days after NMDA (17 nmol) injection on postnatal day 7. Data are [^{3}H]IP$_1$ release (dpm/mg of protein) and presented as the means ± SEM. n = number of assays. Data of the untreated control rat pups are not shown here.
[a] $p < 0.05$; [d] $p < 0.001$; [e] $p < 0.005$, ipsilateral vs. contralateral; two-tailed, paired *t* test.
[b] $p < 0.01$; [c] $p < 0.05$, vs. basal.
[f] $p < 0.05$, vs. QUIS (10^{-5} *M*).

sue. AP4 itself weakly stimulated PPI hydrolysis, but markedly inhibited QUIS stimulation (−60%) in both lesioned and unlesioned hippocampal and striatal tissue (n = 1 assay, data not shown).

Incorporation of *myo*-[^{3}H]inositol into phospholipid after NMDA injection

The extent of incorporation of *myo*-[^{3}H]inositol into membrane phospholipid was examined in three assay runs. Incorporation of *myo*-[^{3}H]inositol into lipids increased significantly in the lesioned hippocampal (by 170%) and striatal (by 90%) tissue compared with the unlesioned tissue (see Fig. 5; lesioned vs. unlesioned, ANOVA). In samples from the lesioned hippocampal tissue incubated with QUIS and CARB, incorporation of *myo*-[^{3}H]inositol into lipid was similar to that in the unstimulated group. However, in the unlesioned hippocampal tissue, QUIS-stimulated *myo*-[^{3}H]inositol was lower (22%) than basal values. In the lesioned striatal slices, radiolabeled *myo*-[^{3}H]-inositol incorporation was decreased significantly (34%) in QUIS-stimulated group when compared with that in the basal group. In the unlesioned striatal tissue, there was no difference in incorporation of *myo*-[^{3}H]inositol into lipid between QUIS, CARB, and basal groups.

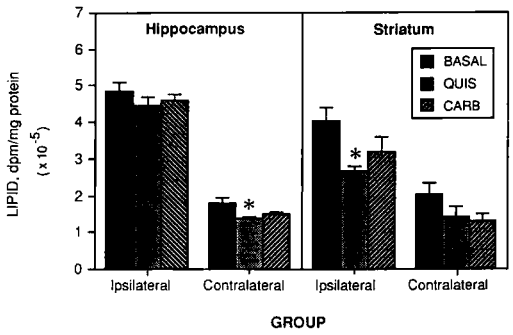

FIG. 5. Incorporation of *myo*-[2-^{3}H]inositol into phospholipid in hippocampal and striatal tissue from animals killed 3 days after NMDA (17 nmol) injection on postnatal day 7. Tissue slices were prepared from hemisphere ipsilateral and contralateral to the injection and incubated with *myo*-[2-^{3}H]inositol and QUIS (10^{-5} *M*), CARB (10^{-2} *M*), or buffer (BASAL) for 2 h (see Materials and Methods for details). After extraction of water-soluble inositol phosphates, 0.2-ml aliquots of the chloroform phase were taken and counted for radioactivity. Values (dpm/mg of protein) are the means ± SEM of triplicate replicates from three separate experiments. In ipsilateral tissue, the incorporation of *myo*-[^{3}H]inositol into phospholipid in all groups is significantly higher than that obtained from contralateral tissue in both hippocampus and striatum ($p < 0.05$), compared with corresponding values of BASAL (post-hoc test, Fisher's PLSD method) after ANOVA.

Influence of TTX and low calcium on QUIS-stimulated PPI hydrolysis

To explore the mechanism for the enhanced QUIS-stimulated PPI hydrolysis after excitotoxic injury, we examined the hypothesis that it is mediated by enhanced release of an endogenous neurotransmitter in the damaged tissue. However, TTX (0.5 μM), which inhibits neurotransmitter release by blocking Na^+ channels at presynaptic terminals (Catterall, 1980), failed to alter QUIS- or CARB-stimulated [^{3}H]IP$_1$ release in damaged or undamaged tissue. Because neurotransmitter release is also sensitive to the extracellular calcium concentration, we assayed [^{3}H]IP$_1$ release in the presence of three different concentrations of extracellular calcium (Fig. 6). In hippocampal tissue from animals killed 3 days after NMDA injection, the magnitude of QUIS-stimulated PPI turnover was further enhanced in the lesioned tissue in low calcium Ca(−) buffer compared with the extent of [^{3}H]IP$_1$ release in Ca(+) buffer (by 59%, n = 5, $p < 0.05$). In the unlesioned hippocampal tissue slices, QUIS-stimulated [^{3}H]IP$_1$ formation in Ca(−) buffer was suppressed by 45%. [^{3}H]IP$_1$ (dpm/mg of protein) stimulated by QUIS was also enhanced in lesioned striatal slices [Ca(−) buffer compared with that in Ca(+) buffer: 259,210 vs. 170,280, n = 5, $p < 0.05$]. In the unlesioned striatal slices, QUIS-stimulated PPI hydrolysis was suppressed by 31% ($p < 0.05$, data not shown). There was no difference between values from

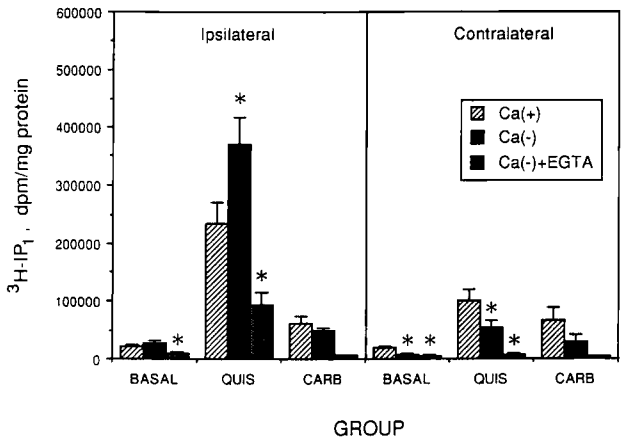

FIG. 6. Comparison of QUIS-stimulated release of [^{3}H]IP$_1$ in hippocampal slices incubated in buffers containing 2.2 m*M* $CaCl_2$, no added $CaCl_2$, or no added $CaCl_2$ plus EGTA. Tissue slices were prepared from the hippocampus of ipsilateral and contralateral hemispheres of NMDA (17 nmol)-injected rats killed on postnatal day 10 and from untreated control rat pups. Slices were washed three times with warm (37°C) buffer in which the $CaCl_2$ was replaced with water, then incubated with QUIS (10^{-5} *M*) or CARB (10^{-2} *M*) or buffer (BASAL) in regular buffer containing 2.2 m*M* $CaCl_2$ [Ca(+)], $CaCl_2$-free buffer [Ca(−)], or $CaCl_2$-free + 0.5 m*M* EGTA [Ca(−) + EGTA] for 2 h. Values (dpm/mg of protein) are the means ± SEM of triplicate results from five different experiments. The stimulation of untreated control rat pups was not different by one-way ANOVA from that of contralateral hippocampal slices and was therefore not shown. CARB-stimulated [^{3}H]IP$_1$ formation in Ca(−) + EGTA buffer was only assayed once in both hemispheres. *$p < 0.05$, comparing [^{3}H]IP$_1$ release in hippocampal tissue incubated in Ca(−) or Ca(−) + EGTA buffer with that in corresponding Ca(+) buffer (post-hoc test, Fisher's PLSD method, after ANOVA).

contralateral and control groups (by one-way ANOVA). In the same experiments, CARB-stimulated PPI hydrolysis in Ca(−) buffer was not increased in any tissue.

In the presence of Ca^{2+}-free buffer, Ca(−) + EGTA, [^{3}H]IP$_1$ formation was markedly suppressed (60–90%) in basal, QUIS, and CARB groups in hippocampus (Fig. 6). A similar degree of repression in PPI hydrolysis in all three groups was also observed in striatal slices (data not shown).

Influence of Ca^{2+} on the incorporation of *myo*-[^{3}H]inositol into phospholipid

The effect of the three different Ca^{2+} concentrations on the incorporation of *myo*-[^{3}H]inositol into phospholipid was assayed (Fig. 7). After incubation in Ca(−) buffer, QUIS-stimulated incorporation of *myo*-[^{3}H]inositol into phospholipid in the lesioned (Fig. 7, Ipsilateral) hippocampal tissue was further increased compared with that in Ca(+) buffer. In the unlesioned (Fig. 7, Contralateral) hippocampal tissue, QUIS- and CARB-stimulated and basal incorporation of *myo*-[^{3}H]inositol into lipid was significantly increased in Ca(−) buffer compared with corresponding values in Ca(+) buffer. After incubation in Ca(−) + EGTA buffer, in the lesioned and unlesioned hippocampal tissue, QUIS-stimulated and basal incorporation of *myo*-[^{3}H]inositol into phospholipid was significantly higher than that of corresponding groups in Ca(+) buffer ($p < 0.05$).

In striatum, the trend of increased incorporation of *myo*-[^{3}H]inositol into lipid in Ca(−) buffer, as well as a further increase in Ca(−) + EGTA, were similar to those in hippocampus ($p < 0.05$, data not shown).

DISCUSSION

In this study, brain injury caused by unilateral injection of NMDA in 7-day-old rats enhanced QUIS-stimulated PPI hydrolysis in the striatum and hippocampus, but did not alter muscarinic-stimulated PPI hydrolysis. The preferential enhancement of QUIS-stimulated PPI hydrolysis appeared on the first day after NMDA injection and persisted for ≤1 month. A similar effect has been reported in neonatal animals after ischemic–hypoxic brain injury (Chen et al., 1988) and in adult rats after kainate-induced hippocampal injury (Nicoletti, 1987).

Pharmacologic studies indicated that the enhancement of PPI turnover after injury was largely restricted to the QUIS receptor. The glutamate agonists Glu and QUIS and the non-NMDA agonist AMPA increased PPI hydrolysis in the lesioned tissue from both hippocampus and striatum, but NMDA was in-

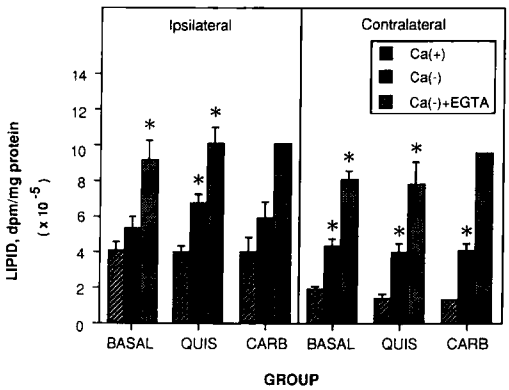

FIG. 7. Incorporation of *myo*-[2-^{3}H]inositol into phospholipid in hippocampal tissue in Ca(+), Ca(−), and Ca(−) + EGTA (see Fig. 6 legend). Hippocampal slices were prepared 3 days after NMDA (17 nmol) injection on postnatal day 7, from ipsilateral and contralateral hemispheres and incubated with *myo*-[2-^{3}H]inositol and QUIS (10^{-5} *M*), CARB (10^{-2} *M*), or buffer (BASAL) in one of three incubation buffers Ca(+), Ca(−), and Ca(−) + EGTA for 2 h. After extraction of water-soluble inositol phosphates, 0.2-ml aliquots of the organic phase were taken and counted for radioactivity (see Materials and Methods for details). Values (dpm/mg of protein) are shown as the means ± SEM of triplicates from five separate experiments. CARB-stimulated labeling into lipid in Ca(−) + EGTA buffer was only assayed once in both hemispheres. *$p < 0.05$, comparing [^{3}H]-labeling into lipid in hippocampal tissue incubated in Ca(−) or Ca(−) + EGTA buffer with that in corresponding Ca(+) buffer (post-hoc test, Fisher's PLSD method, after ANOVA).

effective. QUIS-stimulated PPI hydrolysis was not affected by competitive NMDA antagonists AP7 and CPP or the noncompetitive NMDA antagonist MK-801 at pharmacologically relevant concentrations. However, a very high concentration of MK-801 (10^{-3} *M*) could reduce the response, probably through a nonspecific effect. The finding that AP4 inhibited QUIS stimulation of PPI hydrolysis was consistent with previous results (Schoepp and Johnson, 1988), though its mechanism is still unclear.

These observations are consistent with previous findings that among glutamate receptors, only the QUIS receptor is coupled to PPI hydrolysis in the hippocampus and striatum (Nicoletti et al., 1986*a*). The finding that the AMPA-stimulated PPI turnover was rather low in the unlesioned tissue has also been shown in previous studies (Palmer et al., 1988; Weiss et al., 1988). After brain injury, AMPA-stimulated [^{3}H]IP$_1$ formation could be further enhanced by about onefold in the lesioned tissue when compared with that in the unlesioned tissue. The mechanism of this enhanced AMPA-stimulated PPI hydrolysis is still unclear. It has been suggested that the QUIS receptor can be further subdivided into AMPA-sensitive and non–AMPA-sensitive receptors (Fletcher et al., 1988; Neuman et al., 1988; Palmer et al., 1988). The AMPA-sensitive receptor is coupled to membrane depolarization, whereas the non–AMPA-sensitive receptor may be preferentially coupled to PPI turnover. We also found that the specific AMPA antagonist DNQX failed to block QUIS-stimulated PPI hydrolysis in any tissue. These data may suggest that there is some interaction between the AMPA-sensitive and non–AMPA-sensitive QUIS receptors.

Excitotoxic injury also increased the amount of *myo*-[^{3}H]inositol incorporated into the lipid pool in both hippocampus and striatum, possibly reflecting enhanced lipid turnover of injured membranes. We considered the possibility that this increase in lipid labeling may have contributed to the QUIS-stimulated enhancement of [^{3}H]IP$_1$ release. However, several observations make this unlikely. If the increase in [^{3}H]IP$_1$ was related simply to an increase in the specific activity of radiolabeled phospholipids, an increase in [^{3}H]IP$_1$ release would also be expected in unstimulated injured tissue slices. However, there was no difference in [^{3}H]IP$_1$ release between unstimulated injured and control tissue. Furthermore, QUIS, but not CARB, stimulated [^{3}H]IP$_1$ release in injured tissue with identical elevations in *myo*-[^{3}H]inositol incorporation. Experiments in which QUIS and CARB were added together to the incubation medium indicated that the response was not additive, suggesting that both agonists stimulate the same lipid pool. The data from experiments conducted in low calcium buffer (see below) also suggest that the preferential stimulation of [^{3}H]IP$_1$ release by QUIS is not related to increased lipid labeling. Measurement of *myo*-[^{3}H]inositol–labeled lipids (dpm/mg of protein of the lipid fraction) may not accurately reflect the much smaller pool of phospholipids from which [^{3}H]IP$_1$ is generated.

Several potential mechanisms may be responsible for the specific enhancement of QUIS-stimulated PPI turnover after NMDA-induced injury. An increased number of QUIS receptors coupled to PPI hydrolysis caused by NMDA lesion is one possibility. However, quantitative autoradiography of glutamate binding showed that both QUIS- and NMDA-binding sites decrease in number in NMDA-lesioned tissue (F. S. Silverstein, unpublished observation). This suggests that the number of QUIS- or NMDA-binding sites in these experiments was decreased after the NMDA lesion, and therefore the enhanced QUIS-stimulated PPI turnover observed for the NMDA-lesioned brain tissue is unlikely to be the result of an increased number of QUIS receptors.

A second possibility is that this enhancement might be caused by an increased ability of the QUIS receptor to couple to PLC activity. Recent data suggest that a GTP-binding protein (G protein) links the glutamate receptors to PLC activation (Nicoletti et al., 1988; Ambrosini and Meldolesi, 1989). QUIS and muscarinic receptors appear coupled to PLC by two different G proteins (Ambrosini and Meldolesi, 1989). Because we observed that QUIS-stimulated, but not CARB-stimulated, PPI turnover was enhanced after the NMDA lesion, this enhanced coupling may occur specifically at the QUIS receptor–coupled G protein.

In addition to neurons, large numbers of glia in the tissue slices may possess QUIS receptors that couple to PPI hydrolysis (Pearce et al., 1986). However, after lesioning rats on postnatal day 7, there was relatively little reactive gliosis in NMDA-lesioned tissue on postlesion day 5 (F. S. Silverstein, unpublished observation). Muscarinic receptors on glia are also coupled to PPI hydrolysis (Pearce et al., 1986). However, in the lesioned tissue, CARB addition did not elicit an enhanced stimulation of [^{3}H]IP$_1$ release, suggesting that glia may not contribute to the enhanced QUIS-stimulated PPI hydrolysis in the lesioned tissue during the first week after NMDA injection.

We considered the possibility that QUIS-stimulated potentiation of PPI turnover in the injured tissue was related to increased presynaptic release of endogenous excitatory neurotransmitters. It was hypothesized that decreased release of an inhibitory neurotransmitter might alter the extent of QUIS-stimulated PPI turnover. However, we found that the addition of TTX, which inhibits voltage-sensitive neurotransmitter release, failed to influence the enhancement of QUIS-stimulated PPI hydrolysis. These results suggest that the release of endogenous neurotransmitters does not explain the enhanced QUIS-stimulated PPI turnover observed in NMDA-lesioned tissue.

Experiments in which the calcium concentration of the medium was varied were also performed to ex-

plore possible alterations in neurotransmitter release in the injured slices. Unexpectedly, we found that moderately low calcium [Ca(−), 1 μM] markedly enhanced the effect of injury to increase QUIS-stimulated PPI hydrolysis. In contrast, the Ca(−) buffer attenuated the QUIS- and CARB-stimulated [^{3}H]IP$_1$ release in contralateral and unlesioned striatum and hippocampus. Like the effect of injury itself, augmentation of PPI turnover by low calcium in lesioned tissue was selective for QUIS, but not CARB, stimulation.

Ca(−) buffer also increased incorporation of *myo*-[^{3}H]inositol into total lipids in the lesioned slices and this increase in labeling could contribute to the increased QUIS-stimulated [^{3}H]IP$_1$ formation in the Ca(−) buffer. However, the modest rise in lipid labeling is too small to explain the large increase in PPI turnover. Furthermore, *myo*-[^{3}H]inositol incorporation was greater in Ca(−) than in Ca(+) in the unlesioned hippocampal tissue, but QUIS-stimulated [^{3}H]IP$_1$ release was actually decreased in the same tissue in Ca(−) buffer. This result suggests that increased QUIS-stimulated PPI hydrolysis in Ca(−) buffer was due to enhanced QUIS receptor–coupled events rather than an increased specific activity of the precursor lipid pool. The mechanism for this interesting low calcium effect is unclear. The pronounced reductions observed in [^{3}H]IP$_1$ release in the very low calcium buffer (<10 nM) are consistent with previous reports that PLC is calcium dependent (Gonzales and Crews, 1984; Kendall and Nahorski, 1984; Nicoletti et al., 1986*b*).

NMDA-induced lesions in the neonatal rat brain produce a selective enhancement of QUIS-type EAA-stimulated PPI hydrolysis that is similar to the effect observed in the perinatal ischemic–hypoxic brain injury model (Chen et al., 1988). Our results provide additional evidence that NMDA-induced brain injury replicates important pathophysiologic features of ischemic brain injury. Both types of injury cause a selective, persistent enhancement of EAA-coupled PPI hydrolysis in the developing brain.

Acknowledgment: We thank Dr. Stephen K. Fisher for valuable discussion. This work was supported by Grants NIH P01 NS19613, R01 NS28208, and NS26142 (F.S.S.), and Basic Research Grant 1-910 from the March of Dimes Birth Defects Foundation.

REFERENCES

Ambrosini A. and Meldolesi J. (1989) Muscarinic and quisqualate receptor–induced phosphoinositide hydrolysis in primary cultures of striatal and hippocampal neurons. Evidence for differential mechanism of activation. *J. Neurochem.* **53,** 825–833.

Berridge M. J., Downes C. P., and Hanley M. R. (1982) Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206,** 587–595.

Catterall W. A. (1980) Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annu. Rev. Pharmacol. Toxicol.* **20,** 15–43.

Chen C.-K., Silverstein F. S., Fisher S. K., Statman D., and Johnston M. V. (1988) Perinatal ischemic–hypoxic brain injury enhances quisqualic acid-stimulated phosphoinositide turnover. *J. Neurochem.* **51,** 353–359.

Fisher S. K. and Bartus R. T. (1985) Regional differences in the coupling of muscarinic receptors to inositol phospholipid hydrolysis in guinea pig brain. *J. Neurochem.* **45,** 1085–1095.

Fletcher E. J., Martin D., Aram J. A., Lodge D., and Honore T. (1988) Quinoxalinediones selectively block quisqualate and kainate receptors and synaptic events in rat neocortex and hippocampus and frog spinal cord *in vitro*. *Br. J. Pharmacol.* **95,** 585–597.

Gonzales R. A. and Crews F. T. (1984) Characterization of the cholinergic stimulation of phosphoinositide hydrolysis in rat brain slices. *J. Neurosci.* **4,** 3120–3127.

Heacock A. J., Fisher S. K., and Agranoff B. W. (1987) Enhanced coupling of neonatal muscarinic receptors in rat brain to phosphoinositide turnover. *J. Neurochem.* **48,** 1904–1911.

Ikonomidou C., Mosinger J. L., Shahid Salles K., Labruyere J., and Olney J. W. (1989) Sensitivity of the developing rat brain to hypobaric/ischemic damage parallels sensitivity to *N*-methyl-D-aspartate neurotoxicity. *J. Neurosci.* **9,** 2809–2818.

Kendall D. A. and Nahorski S. R. (1984) Inositol phospholipid hydrolysis in rat cerebral cortical slices: II. Calcium requirement. *J. Neurochem.* **42,** 1388–1394.

Lowry O. H., Rosebrough N. J., Farr A. L., and Randall R. J. (1951) Protein measurements with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

McDonald J. W., Silverstein F. S., and Johnston M. V. (1988) Neurotoxicity of *N*-methyl-D-aspartate is markedly enhanced in developing rat central nervous system. *Brain Res.* **459,** 200–203.

Neuman R. S., Ben-Ari Y., and Cherubini E. (1988) Antagonism of spontaneous and evoked bursts by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the CA3 region of the in vitro hippocampus. *Brain Res.* **474,** 201–203.

Nicoletti F., Iadarola M. J., Wroblewski J. T., and Costa E. (1986*a*) Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: developmental changes and interaction with α_1-adrenoceptors. *Proc. Natl. Acad. Sci. USA* **83,** 1931–1935.

Nicoletti F., Wroblewski J. T., Novelli A., Alho H., Guidotti A., and Costa E. (1986*b*) The activation of inositol phospholipid metabolism as a signal transducing system for excitatory amino acids in primary cultures of cerebellar granule cells. *J. Neurosci.* **6,** 1905–1911.

Nicoletti F., Wroblewski J. T., Alho H., Eva C., Fadda E., and Costa E. (1987) Lesions of putative glutamatergic pathways potentiate the increase of inositol phospholipid hydrolysis elicited by excitatory amino acids. *Brain Res.* **436,** 103–112.

Nicoletti F., Wroblewski J. T., Fadda E., and Costa E. (1988) Pertussis toxin inhibits signal transduction at a specific metabolotropic glutamate receptor in primary cultures of cerebellar granule cells. *Neuropharmacology* **27,** 551–556.

Palmer E., Monaghan D. T., and Cotman C. W. (1988) Glutamate receptors and phosphoinositide metabolism: stimulation via quisqualate receptors is inhibited by *N*-methyl-D-aspartate receptor activation. *Mol. Brain Res.* **4,** 161–165.

Pearce B., Albrecht J., Morrow C., and Murphy S. (1986) Astrocyte glutamate receptor activation promotes inositol phospholipid turnover and calcium flux. *Neurosci. Lett.* **72,** 335–340.

Schoepp D. D. and Johnson B. G. (1988) Excitatory amino acid agonist–antagonist interactions at 2-amino-4-phosphonobutyric acid–sensitive quisqualate receptors coupled to phosphoinositide hydrolysis in slices of rat hippocampus. *J. Neurochem.* **50,** 1605–1613.

Weiss S., Schmidt B. H., Sebben M., Kemp D. E., Bockaert J., and Sladeczek F. (1988) Neurotransmitter-induced inositol phosphate formation in neurons in primary culture. *J. Neurochem.* **50,** 1425–1433.