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INHIBITION OF INOSITOL PHOSPHOLIPID SYNTHESIS AND NOREPINEPHRINE-STIMULATED HYDROLYSIS IN RAT BRAIN SLICES BY EXCITATORY AMINO ACIDS

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Abstract—Inhibition by excitatory amino acid agonists of norepinephrine (NE)-stimulated phosphoinositide hydrolysis was studied in rat brain slices. Inhibition was not observed in cortical slices prelabeled with [^{3}H]inositol but was observed when slices were incubated simultaneously with [^{3}H]inositol, glutamate, and NE. Therefore, we hypothesized that glutamate inhibits the synthesis of inositol phospholipids available to the α_1-adrenergic receptor, thereby reducing NE-stimulated phosphoinositide hydrolysis. To test this hypothesis, the distribution of [^{3}H]inositol in cortical slices was measured after 5, 10, 20, 40 and 60 min of incubation, with some slices being exposed to 200 μM NE, 1 mM glutamate, 1 mM *N*-methyl-D-aspartate (NMDA), 1 mM kainate, 1 mM quisqualate, or to NE in the presence of each of the excitatory amino acid agonists. Glutamate had little effect on the slice content of free [^{3}H]inositol, but it severely reduced the synthesis of [^{3}H]inositol phospholipids, in the presence or absence of NE. Glutamate also abolished NE-induced production of [^{3}H]inositol monophosphate, [^{3}H]inositol bisphosphate and [^{3}H]inositol trisphosphate. Quisqualate mimicked the effects of glutamate, whereas NMDA and kainate caused less inhibition of the synthesis of [^{3}H]inositol phospholipids and did not inhibit the response to NE. Glutamate produced similar inhibitory effects in slices from hippocampus and striatum. To test if the inhibitory effect of glutamate was the result of irreversible cell damage, cortical slices were incubated with 1 mM glutamate for 60 min prior to exposure to [^{3}H]inositol and NE. Preincubation with glutamate did not reduce the synthesis of [^{3}H]inositol phospholipids or inhibit NE-stimulated [^{3}H]inositol monophosphate production. These results indicate that glutamate impairs the synthesis of inositol phospholipids. Each of the excitatory amino acid agonists, quisqualate, NMDA and kainate, inhibited [^{3}H]inositol phospholipid synthesis, but only quisqualate affected [^{3}H]inositol phospholipids available to the α_1-adrenergic receptor.

One of the major mechanisms by which neurotransmitters induce postsynaptic responses appears to be by stimulating the hydrolysis of lipids. The clearest model of this type of mechanism is the receptor-coupled activation of a guanine nucleotide binding protein which stimulates phospholipase C to hydrolyze inositol-containing phospholipids [1, 2]. At least two second messengers are formed from this reaction, diacylglycerol which modulates the activity of protein kinase C, and inositol trisphosphate which modulates the intracellular concentration of calcium [3, 4].

Regulatory mechanisms associated with the phosphoinositide second messenger system are only beginning to be identified. Because of our interest in the mechanisms producing status epilepticus [5, 6] and because of the increasing evidence that excitatory amino acids are important mediators of many types of seizures [7], we were especially interested in recent reports describing modulatory effects of excitatory amino acids on cholinergic or adrenergic receptor-coupled phosphoinositide hydrolysis in CNS tissues [8–10]. Baudry *et al.* [8] and Schmidt *et al.* [9] reported that excitatory amino acids inhibit phosphoinositide hydrolysis stimulated by carbachol but not by norepinephrine (NE). Nicoletti *et al.* [10] reported that excitatory amino acids inhibit phosphoinositide hydrolysis stimulated by NE but not by carbachol. The contrasting effects reported in these studies may be due to differences of the methods and tissues used in each laboratory. In the present study, we compared different methods used to measure the modulation by glutamate of NE-induced phosphoinositide hydrolysis. From our initial results, we formulated the hypothesis that at least a part of the inhibition of NE-induced phosphoinositide hydrolysis caused by excitatory amino acids was due to inhibition of the incorporation of [^{3}H]inositol into [^{3}H]phosphoinositides available to α_1-adrenergic receptors. We also compared the effects of glutamate on slices prepared from rat cerebral cortex, hippocampus and striatum and identified a quisqualate-selective site as being responsible for the inhibitory effects of glutamate on the response to NE in cortical slices.

MATERIALS AND METHODS

Tissue preparation. Male, Sprague–Dawley rats (200–250 g) were decapitated, and the brains were removed rapidly and placed in ice-cold 0.32 M sucrose for dissection on ice. Slices (0.3 × 0.3 mm)

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were prepared from isolated cerebral cortex, hippocampus or striatum using a McIlwain tissue slicer. Slices were washed several times in incubation medium (NaCl, 122 mM; $NaHCO_3$, 3.6 mM; $MgCl_2$, 1.2 mM; KCl, 5 mM; $CaCl_2$, 1.3 mM; *N*-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid (HEPES), 30 mM; glucose, 11 mM; freshly bubbled with 95% O_2/5% CO_2; adjusted to pH 7.4) and were regenerated by incubation for 1 hr at 37° followed by several washes with fresh medium.

Assay of [^{3}H]inositol incorporation into slices and [^{3}H]inositol monophosphate production. Several different experimental protocols were used to expose slices to [^{3}H]inositol and to test the effects of several drugs. Data shown in Table 1 are from experimental procedures unique to that table and the methods are described in the table legend. In all experiments, 50-μl samples of gravity-packed cortical slices (containing approximately 1–2 mg protein) or 25-μl samples of hippocampal or striatal slices were used and all experiments were carried out in triplicate.

Data in Figs 1–5 are from experiments using the following procedures, which were adapted from previous reports [11–15], in which slices were exposed simultaneously to [^{3}H]inositol and test agents. Aliquots of slices were incubated in a final volume of 500 μl of incubation medium containing 10 mM LiCl, 0.5 μM *myo*-[2-^{3}H]inositol (15 Ci/mmol) and the indicated concentrations of test agents for 5, 10, 20, 40 or 60 min at 37°. At the end of the incubation, samples were rapidly washed two times with 3 ml of ice-cold medium and were mixed with 1.5 ml of $CHCl_3$:MeOH:12 N HCl (1:2:0.01). The samples were incubated at room temperature for 30 min and then transferred to extraction tubes and mixed with 1 ml of $CHCl_3$ and 0.5 ml of H_2O. Phases were separated by centrifugation. Aqueous fractions were transferred to clean tubes and mixed with 0.5 ml of a 50% slurry of AG 1-X8 resin. After 10 min, the mixtures were poured over small columns, and the [^{3}H]inositol and [^{3}H]inositol phosphate fractions were eluted sequentially and collected in scintillation vials as described previously [11, 16]. The radioactivity in each sample was measured with a counting efficiency of 40%. Organic fractions were transferred to scintillation vials and dried overnight at room temperature, and the radioactivity was measured.

For experiments reported in Fig. 6, after the regenerative incubation and subsequent washes, slices were incubated in medium containing no addition, 200 μM NE, 1 mM glutamate, or NE and glutamate for 60 min. Slices were then washed several times in fresh medium. Slices were added to vials containing [^{3}H]inositol and LiCl, as described above, and incubated for 60 min with the additions indicated. Samples were then analyzed exactly as described above.

Statistical analysis. Data were analyzed using Student's *t*-test, and a P value of < 0.05 was considered significant.

Materials. AG 1-X8 anion exchange resin was obtained from Bio-Rad (Richmond, CA). l-Norepinephrine, l-glutamate, *N*-methyl D-aspartate (NMDA) and quisqualate were from the Sigma Chemical Co. (St. Louis, MO). American Radiolabelled Chemicals, Inc. (St. Louis, MO) supplied the *myo*-[2-^{3}H(N)]inositol (15 Ci/mmol).

RESULTS

In the first experiments of this project, we compared two methods of measuring receptor-coupled inositol phospholipid hydrolysis in cortical slices incubated in the presence of NE and/or glutamate (Table 1). With the first method, slices were prelabeled with [^{3}H]inositol for 1 hr and then washed several times to remove exogenous [^{3}H]inositol. Subsequent incubation with NE and/or glutamate resulted in no significant inhibition by glutamate (10% inhibition) of NE-induced [^{3}H]inositol mono-

Table 1. Modulation by glutamate of NE-induced [^{3}H]inositol monophosphate production

	[^{3}H]Inositol monophosphate (cpm)	
Addition	Method 1	Method 2
None	1090 ± 115	1380 ± 83
Glutamate	1260 ± 121	840 ± 61
NE	3870 ± 229	4680 ± 415
NE and glutamate	3480 ± 248	2450 ± 87*

Two different methods were used to measure the effect of glutamate (1 mM) on [^{3}H]inositol monophosphate production stimulated by NE (200 μM) in cortical slices incubated for 1 hr at 37°. Method 1: Slices were incubated with [^{3}H]inositol for 1 hr in the absence of $CaCl_2$, which results in enhanced synthesis of [^{3}H]inositol phospholipids [16]. Subsequently, the slices were thoroughly washed to remove free exogenous [^{3}H]inositol, and aliquots of slices (50 μl) were incubated for 1 hr in medium (450 μl) containing $CaCl_2$ and NE, glutamate or both agents. Method 2: Slices (50 μl) were added to vials containing [^{3}H]inositol and incubated in incubation medium (without $CaCl_2$) in a final volume of 500 μl for 1 hr. Subsequently, $CaCl_2$ (1.3 mM), NE and/or glutamate were added to the vials, and the incubation was continued for another hour. Values are means ± SEM from three experiments measured in triplicate.

* $P < 0.05$ compared with NE (Student's *t*-test).

phosphate production. In contrast, with Method 2 slices were preincubated with [^{3}H]inositol and then NE and/or glutamate were added without removing the [^{3}H]inositol. Using this method, glutamate significantly reduced the NE-induced [^{3}H]inositol monophosphate production (Table 1).

These results led us to hypothesize that one effect of glutamate may be impairment of the availability of [^{3}H]inositol phospholipids to the α_1-adrenergic receptor. Therefore, preincubation with [^{3}H]inositol was deleted to study in detail the time course of [^{3}H]inositol distribution when slices were incubated in the presence of NE and/or excitatory amino acid agonists. A similar protocol of simultaneously adding [^{3}H]inositol and agonists to medium with brain slices has been used previously by a number of investigators studying agonist-induced phosphoinositide hydrolysis [11–15].

Free [^{3}H]inositol. We first tested whether excitatory amino acids inhibited the uptake of [^{3}H]inositol into cortical slices by measuring the free [^{3}H]inositol and the total [^{3}H] throughout 5–60 min of incubation. Figure 1 shows the content of [^{3}H]inositol in cortical slices incubated for 5, 10, 20, 40 or 60 min in the presence of 0.5 μM [^{3}H]inositol. [^{3}H]Inositol rapidly accumulated in the slices during the first 5 min of incubation. In the absence of added agents, the initial rapid accumulation of free [^{3}H]inositol was followed by a much slower increase between 5 and 60 min (Fig. 1A). During the shorter time periods, there were no significant effects of NE, glutamate, quisqualate or NMDA on the content of [^{3}H]inositol. However, in the presence of NE the secondary slow increase of [^{3}H]inositol was not observed and the slice [^{3}H]inositol concentration remained approximately constant between 5 and 60 min of incubation (Fig. 1B). This resulted in significantly less free [^{3}H]inositol in the slices after 40 and 60 min of incubation with NE compared with no addition (Fig. 1A). After 60 min of incubation, glutamate and quisqualate also significantly reduced the slice [^{3}H]-inositol concentration compared with slices incubated without added agents. Figure 1B shows that in the presence of NE the excitatory amino acids did not alter significantly [^{3}H]inositol in the slices compared with incubation with NE alone. Both with and without NE, kainate had effects similar to those of NMDA (data not shown). Thus, in the presence of NE, the excitatory amino acids did not modify the free [^{3}H]inositol in cortical slices.

Total [^{3}H]. During the incubation, [^{3}H]inositol is converted to a variety of products, the major ones

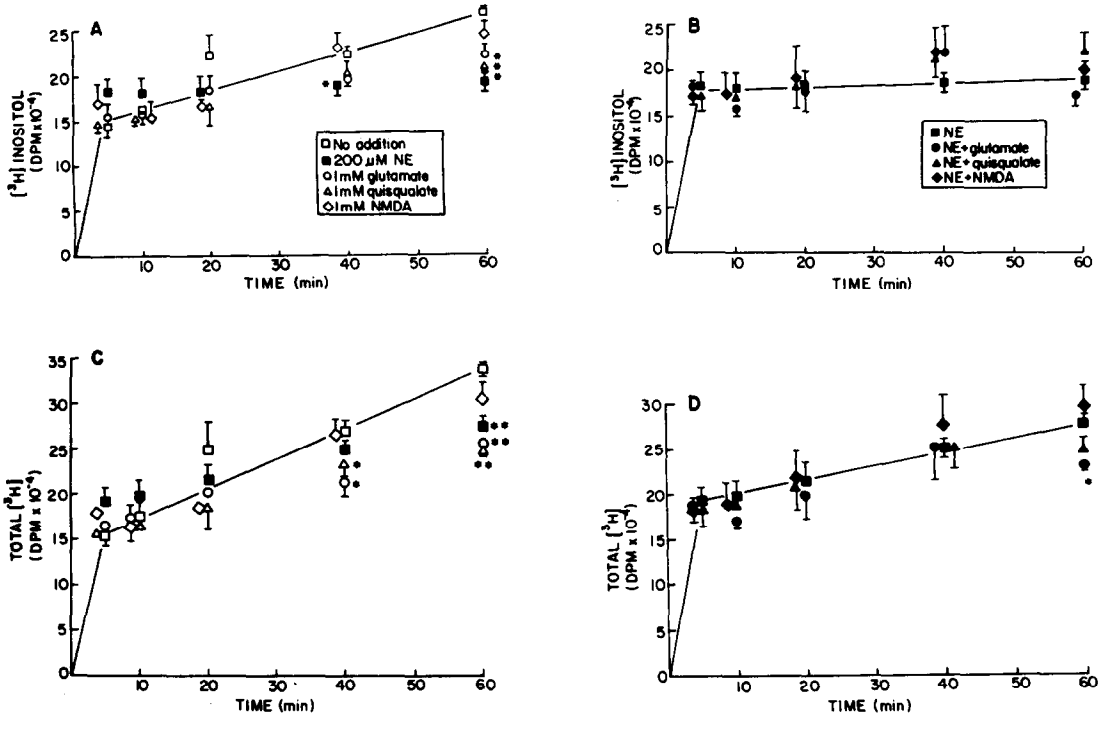

Fig. 1. Free [^{3}H]inositol (A,B) and total [^{3}H] (C,D) in cortical slices. After preincubation for 60 min, slices were washed and incubated for 5, 10, 20, 40 or 60 min with 0.5 μM [^{3}H]inositol as described in Materials and Methods. Panels A and B show the slice content of free [^{3}H]inositol and panels C and D show the total [^{3}H] in the slices, which is the sum of free [^{3}H]inositol, [^{3}H]inositol phospholipids and [^{3}H]inositol monophosphate, the three fractions that account for most of the [^{3}H] in cortical slices. Panels A and C show the effects of each agent added individually compared with (□) no addition; (■) 200 μM NE; (○) 1 mM glutamate; (△) 1 mM quisqualate; and (◇) 1 mM NMDA. Panels B and D show the effect of (■) 200 μM NE compared with each agent in the presence of 200 μM NE: (●) NE and glutamate; (▲) NE and quisqualate; and (◆) NE and NMDA. Each symbol represents the mean ± SEM of three experiments measured in triplicate. Key: (*) $P < 0.05$; and (**) $P < 0.005$, compared with no addition in A and C or compared with NE in B and D at each time point (Student's *t*-test).

being [^{3}H]inositol phospholipids and [^{3}H]inositol monophosphate (since 10 mM LiCl is included in the incubation medium to inhibit inositol-1-phosphatase). Panels C and D of Fig. 1 show the total accumulation of [^{3}H]inositol-containing products in cortical slices as a function of time and show the effects of NE and of the excitatory amino acids. In the absence of added agents there was a linear increase with time of total [^{3}H] from 5 to 60 min which was due largely (67%) to the increased free [^{3}H]inositol in the slices (as shown in Fig. 1A). In contrast, in the presence of NE, glutamate or quisqualate there was a slower rate of increase of total [^{3}H] in the slices. In the presence of NE, the increase was due largely (80%) to [^{3}H]inositol phospholipids. Glutamate and quisqualate resulted in small decreases of total [^{3}H] compared with basal at 40 and 60 min (Fig. 1C), but in the presence of NE the excitatory amino acids did not reduce the total [^{3}H] significantly except for a slight reduction by glutamate at 60 min (Fig. 1D). This is one indication that under these conditions the excitatory amino acids did not appear to induce cell lysis. In data presented later in this report, the consistent slice content of total [^{3}H] was used in some cases to normalize data to compensate for variability of aliquoting slices into incubation vials.

[^{3}H]Inositol phospholipids. Figure 2 shows the rate of incorporation of [^{3}H]inositol into phospholipids in cortical slices. In the absence of added agents, the rate of synthesis of [^{3}H]inositol phospholipids was linear throughout 60 min of incubation. NE induced a slight increase of [^{3}H]inositol phospho-

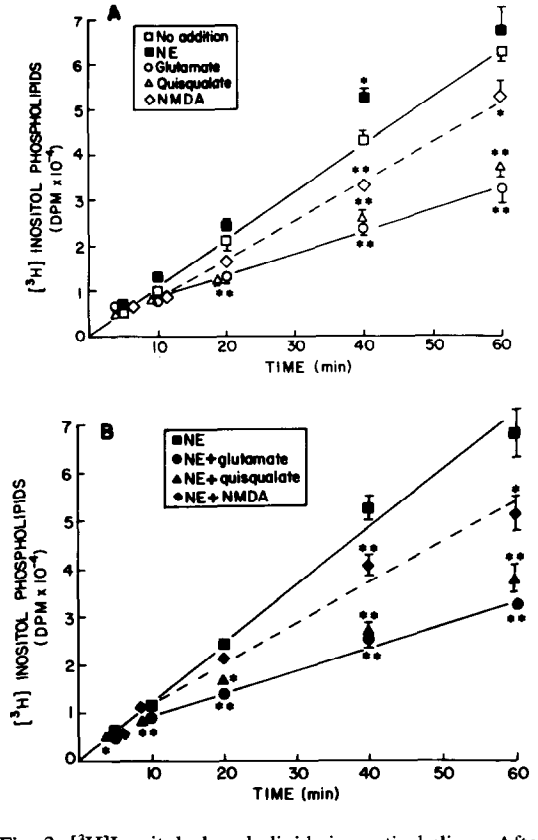

Fig. 2. [^{3}H]Inositol phospholipids in cortical slices. After preincubation for 60 min, slices were washed and incubated for 5, 10, 20, 40 or 60 min with [^{3}H]inositol followed by measurement of radioactivity in the lipid fraction. Panels A and B show the rate of incorporation of [^{3}H]inositol into [^{3}H]inositol phospholipids. Panel A shows the effects of each agent alone compared with (□) no addition; (■) 200 μM NE; (○) 1 mM glutamate; (△) 1 mM quisqualate; and (◇) 1 mM NMDA. Panel B shows the effect of (■) 200 μM NE compared with each agent in the presence of 200 μM NE; (●) NE and glutamate; (▲) NE and quisqualate; and (◆) NE and NMDA. Each symbol represents the mean ± SEM of three experiments measured in triplicate. Key: (*) $P < 0.05$; and (**) $P < 0.005$ compared with no addition in panel A or compared with 200 μM NE in panel B at each time point (Student's *t*-test).

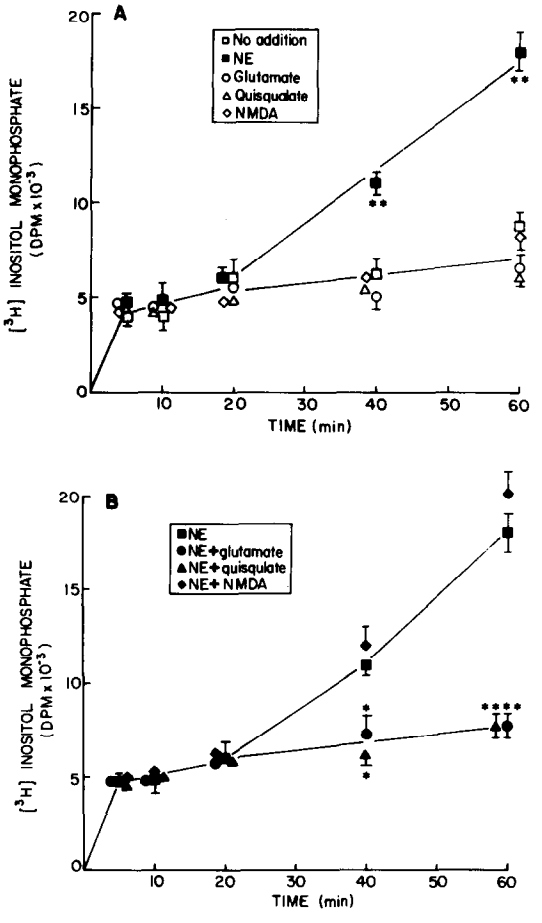

Fig. 3. [^{3}H]Inositol monophosphate production in cortical slices. After preincubation for 60 min, slices were washed and incubated for 5, 10, 20, 40 or 60 min with [^{3}H]inositol. Panel A shows the formation of [^{3}H]inositol monophosphate with the following additions to the incubation medium: (□) none; (■) 200 μM NE; (○) 1 mM glutamate; (△) 1 mM quisqualate; and (◇) 1 mM NMDA. Panel B shows the effects of excitatory amino acids on NE-induced production of [^{3}H]inositol monophosphate: (■) NE; (●) NE and glutamate; (▲) NE and quisqualate; and (◆) NE and NMDA. Each symbol represents the mean ± SEM of three experiments measured in triplicate. Key: (*) $P < 0.05$; and (**) $P < 0.005$ compared at each time point with no addition in panel A or with NE in panel B (Student's *t*-test).

lipids which was statistically significant only at 40 min (Fig. 2A). Glutamate and quisqualate significantly inhibited the synthesis of [^{3}H]inositol phospholipids at 20, 40 and 60 min of incubation. NMDA caused a smaller inhibition of [^{3}H]inositol phospholipid synthesis than did quisqualate.

Figure 2B compares the rate of synthesis of [^{3}H]inositol phospholipids in the presence of NE to the rates observed with NMDA, glutamate or quisqualate in addition to NE. Quisqualate reduced significantly the synthesis of [^{3}H]inositol phospholipids in the presence of NE at every time period tested from 5 to 60 min. Glutamate produced an inhibitory effect similar to that of quisqualate, whereas NMDA resulted in much less inhibition which was statistically significant only at 40 and 60 min. The effects of kainate were similar to those of NMDA (data not shown).

[^{3}H]Inositol monophosphate. Figure 3 shows the time course of the production of [^{3}H]inositol monophosphate. In all samples there was already significant [^{3}H]inositol monophosphate present at 5 min which we presume is due to a contaminant and not derived from receptor-coupled phosphoinositide hydrolysis. NE significantly stimulated the production of [^{3}H]inositol monophosphate after 40 and 60 min of incubation, whereas the excitatory amino acids had no significant effects compared with no addition (Fig. 3A). The delay of the production of [^{3}H]inositol monophosphate in the presence of NE presumably reflects the time required to label receptor-coupled inositol phospholipids to a sufficient degree to obtain measurable [^{3}H]inositol monophosphate. Using a similar protocol, a 40-min lag in the production of [^{3}H]inositol monophosphate induced by cholinergic agonists in brain slices has been reported [12].

Figure 3B shows the effects of the excitatory amino acids on NE-induced [^{3}H]inositol monophosphate production. NMDA did not alter the effect of NE, whereas glutamate and quisqualate totally blocked the stimulatory effect of NE. Taken in conjunction with the results shown in Figs 1 and 2, these results suggest that glutamate and quisqualate inhibit the incorporation of [^{3}H]inositol into phospholipids in adrenergic receptor-containing cells. In contrast, because NMDA reduced the synthesis of [^{3}H]inositol phospholipids, but not the production of [^{3}H]inositol monophosphate stimulated by NE, NMDA apparently inhibits inositol phospholipid synthesis in other cells or in compartments not coupled to the α_1-adrenergic receptor.

[^{3}H]Inositol polyphosphates. Results similar to those obtained with [^{3}H]inositol monophosphate were found by measuring [^{3}H]inositol bisphosphate and [^{3}H]inositol trisphosphate (Fig. 4). NE stimulated the production of each product, and glutamate and quisqualate effectively inhibited the response to NE, but NMDA had no significant effect.

Hippocampus and striatum. The regional specificity of the inhibitory effect of glutamate on the synthesis of [^{3}H]inositol phospholipids and on NE-induced [^{3}H]inositol monophosphate production was examined by carrying out experiments the same as those reported for cortical slices using slices from the hippocampus and the striatum (Fig. 5). In both

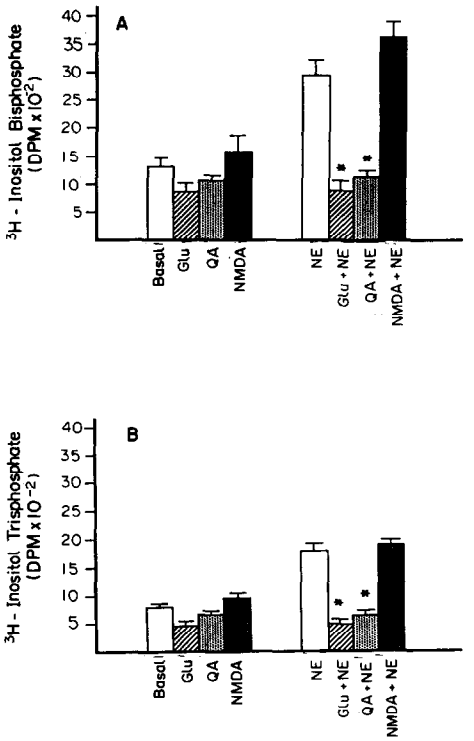

Fig. 4. [^{3}H]Inositol bisphosphate and [^{3}H]inositol trisphosphate in cortical slices. Slices were preincubated for 60 min, washed, and incubated for 60 min with [^{3}H]inositol and the indicated agents. Each value is the mean ± SEM of three experiments measured in triplicate. Key: (*) $P < 0.05$ compared with NE (Student's *t*-test).

regions, glutamate inhibited the synthesis of [^{3}H]inositol phospholipids both in the absence and the presence of NE (Fig. 5, panels A and C). Glutamate also effectively blocked NE-stimulated [^{3}H]inositol monophosphate production in slices from both regions (Fig. 5, panels B and D).

Glutamate pretreatment. Glutamate is a neurotoxic agent and it could reduce the incorporation of [^{3}H]inositol into lipids by causing cell lysis. To test this possibility, an additional preincubation was added to the protocol in which equal aliquots of slices were incubated under one of four different conditions for 1 hr at 37°: control medium, medium with 200 μM NE, medium with 1 mM glutamate, or medium with NE and glutamate. After this treatment, the slices were washed thoroughly and then incubated for 1 hr in medium containing [^{3}H]inositol and NE and/or glutamate (as described in Figs 1–3). Figure 6 shows the results from these experiments. The primary observation was that, regardless of the preincubation condition, NE produced a significant stimulation of [^{3}H]inositol monophosphate production. Therefore, incubation for 1 hr with glutamate did not cause cell damage resulting in the total inhibition of NE-stimulated [^{3}H]inositol monophosphate production that was demonstrated in Fig. 3. This supports the hypothesis that the inhibitory effect of glutamate is due to inhibition of the synthesis of [^{3}H]inositol phospho-

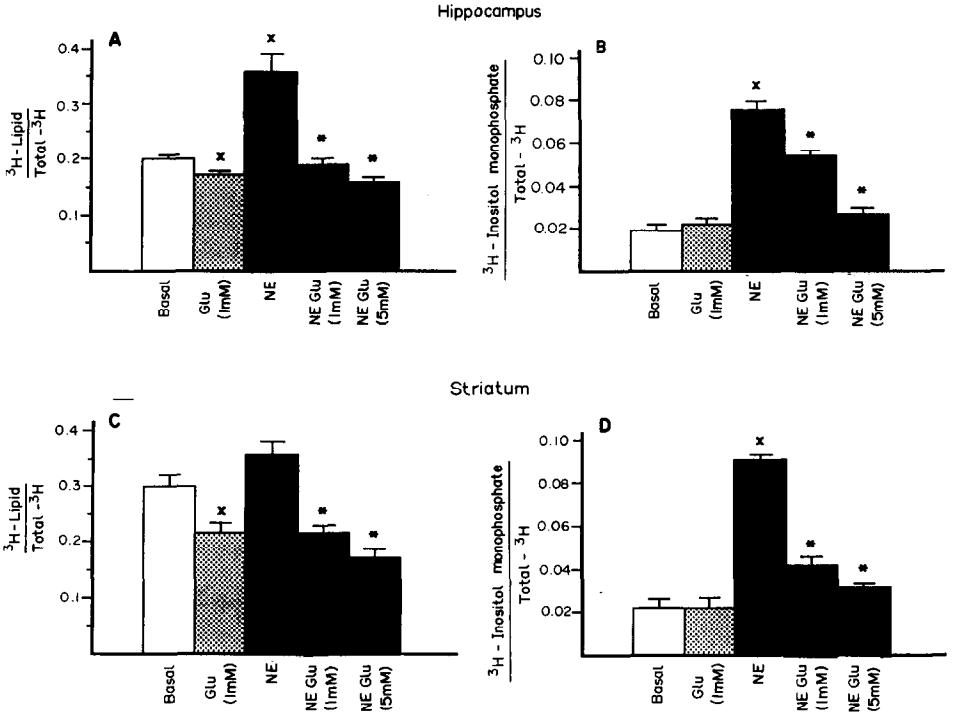

Fig. 5. Effects of excitatory amino acids on [^{3}H]inositol phospholipid synthesis and on NE-induced production of [^{3}H]inositol monophosphate in slices from hippocampus (A,B) and striatum (C,D). After preincubation for 60 min, slices were washed and incubated for 60 min with [^{3}H]inositol and the agents indicated as described in Materials and Methods. Each value is the mean ± SEM of three experiments measured in triplicate. Key: (×) $P < 0.05$ compared with basal (no addition); and (*) $P < 0.05$ compared with 200 μM NE (Student's t-test).

lipids and not to cell damage or inhibition of the activation of the α_1-adrenergic receptor by NE.

DISCUSSION

The first major finding of this investigation was that the synthesis of [^{3}H]inositol phospholipids in rat brain slices was inhibited by glutamate and by each of the selective excitatory amino acid agonists, quisqualate, NMDA and kainate. This inhibitory effect was shown not to be due to inhibition of the uptake of [^{3}H]inositol, but occurred at a step in the pathway incorporating it into phospholipids. The precise site of action has yet to be determined. Glutamate and quisqualate more effectively inhibited [^{3}H]inositol phospholipid synthesis than did NMDA or kainate. For example, Fig. 2A showed that after 60 min of incubation quisqualate had inhibited the synthesis of [^{3}H]inositol phospholipids by 50% while NMDA inhibited it by 15%, compared with basal conditions. In the presence of NE, the percentage inhibition by quisqualate was even greater. Because more than 85% of the incorporated [^{3}H]inositol has been reported to be in phosphatidylinositol in brain tissue, and less than 15% in the polyphosphoinositides [15, 17–19], it is apparent that the major inhibitory effects of glutamate and quisqualate occur at a step prior to the synthesis of phosphatidylinositol.

A second important finding in this report is that the reduced synthesis of [^{3}H]inositol phospholipids caused by glutamate or quisqualate, but not that caused by NMDA or kainate, resulted in inhibition of NE-induced production of [^{3}H]inositol phosphates. Thus, the inhibitory effects of the selective

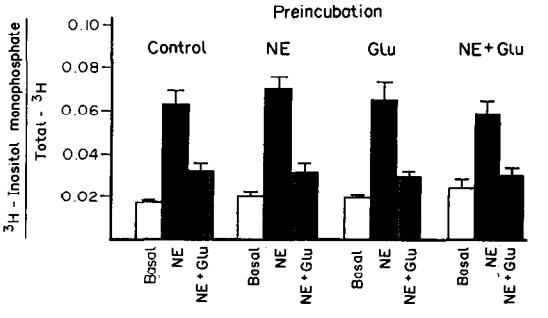

Fig. 6. Effects of preincubation with NE and/or glutamate on NE-stimulated [^{3}H]inositol monophosphate production. Cortical slices were preincubated for 1 hr at 37° in medium followed by several washes. Slices were then preincubated a further 1 hr in medium containing no addition (control), 200 μM NE, 1 mM glutamate (glu) or NE and glutamate. Afterwards, slices were thoroughly washed and incubated in medium containing [^{3}H]inositol with: no addition (basal), 200 μM NE, or NE and 1 mM glutamate. Each value is the mean ± SEM of three experiments measured in triplicate.

agonists are cell-specific, and this specificity indicates a colocalization of quisqualate-sensitive sites and α_1-adrenergic receptors. In clear contrast, although NMDA significantly reduced the synthesis of [^{3}H]inositol phospholipids, it did not alter NE-induced production of [^{3}H]inositol phosphates. This indicates that the α_1-adrenergic receptor and the NMDA-sensitive site are either not colocalized or that the pool of phosphoinositides affected by NMDA is not coupled to the α_1-adrenergic receptor. The same result was obtained with kainic acid as was reported for NMDA.

An alternative explanation for these results may be that the excitatory amino acid agonists stimulate a massive release of free inositol from phospholipids, resulting in a large dilution of the added [^{3}H]inositol with unlabeled inositol. However, this effect would also result in depletion of inositol phospholipids, and the end result may be identical. This possibility remains to be investigated, but the lack of a significant decrease of free [^{3}H]inositol in the presence of the excitatory amino acid agonists suggests that this did not occur.

The observed inhibition of [^{3}H]inositol phospholipid synthesis theoretically could be due to cell lysis caused by the excitatory amino acid agonists since these agents are well known to be neurotoxic agents. To test this possibility, slices were first incubated for 60 min with glutamate under conditions identical to those that were used to measure the synthesis of [^{3}H]inositol phospholipids. It was shown that, subsequent to this treatment, slices were able to synthesize [^{3}H]inositol phospholipids and the system responded to NE identically as did slices preincubated without glutamate. Removal of chloride from the incubation medium also did not alter the inhibitory effect of glutamate (data not shown). Therefore, the conditions employed in these experiments do not appear to lead to irreversible cell damage affecting the NE-responsive phosphoinositide system.

These results may help to explain the three apparently contradictory reports in the literature concerning the effects of excitatory amino acid agonists on NE-induced [^{3}H]inositol phosphate production. Nicoletti *et al.* [10] observed inhibition of the NE-response employing an assay in which [^{3}H]inositol, NE and excitatory amino acid agonists were present together for 60 min. In contrast, Baudry *et al.* [8] and Schmidt *et al.* [9] prelabeled tissue and then removed the exogenous [^{3}H]inositol before adding NE and other agonists. Therefore, in the latter two studies no inhibition was observed, because the inositol phospholipids were labeled prior to addition of glutamate. Using their methods, submaximal labeling of the adrenergic receptor-coupled phosphoinositides or longer incubation times with NE and glutamate may result in reduced production of [^{3}H]inositol phosphates if the [^{3}H]inositol phospholipids are depleted and resynthesis from [^{3}H]inositol is inhibited by glutamate. The results in each of the previous reports are consistent with our observations that at least one mechanism by which the excitatory amino acids inhibit NE-induced phosphoinositide hydrolysis is due to the reduction of the availability of the lipid substrates.

Whether or not NE-induced phosphoinositide hydrolysis is modulated by excitatory amino acids *in vivo* will require further investigations. Our results suggest that glutamate will have its major effects when its synaptic concentration remains elevated for long enough to significantly deplete phosphoinositides available to α_1-adrenergic receptors. This, in turn, would be dependent upon the rate at which these receptors are activated. These conditions might be most likely to occur in conditions associated with neurological disorders, such as seizures. Also, phosphoinositide depletion induced by glutamate may play a role in the neurotoxic properties of the excitatory amino acids.

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REFERENCES

1. Fisher SK and Agranoff BW, Receptor activation and inositol lipid hydrolysis in neural tissues. *J Neurochem* **48**: 999–1017, 1987.
2. Abdel-Latif AA, Calcium-mobilizing receptors, polyphosphoinositides, and the generation of second messengers. *Pharmacol Rev* **38**: 227–272, 1986.
3. Berridge MJ, Inositol trisphosphate and diacylglycerol: Two interacting second messengers. *Ann Rev Biochem* **56**: 159–193, 1987.
4. Majerus PW, Connolly TM, Deckmyn H, Ross TS, Bross TE, Ishii H, Bansal VS and Wilson DB, The metabolism of phosphoinositide-derived messenger molecules. *Science* **234**: 1519–1526, 1986.
5. Morrisett RA, Jope RS and Snead OC, Status epilepticus is produced by administration of cholinergic agonists to lithium-treated rats: Comparison with kainic acid. *Exp Neurol* **98**: 594–605, 1987.
6. Jope RS, Simonato M and Lally K, Acetylcholine content in rat brain is elevated by status epilepticus induced by lithium and pilocarpine. *J Neurochem* **49**: 944–951, 1987.
7. Ben-Ari Y, Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14**: 375–403, 1985.
8. Baudry M, Evans J and Lynch G, Excitatory amino acids inhibit stimulation of phosphatidylinositol metabolism by aminergic agonists in hippocampus. *Nature* **319**: 329–331, 1986.
9. Schmidt BH, Weiss S, Sebben M, Kemp DE, Bockaert J and Sladeczk F, Dual action of excitatory amino acids on the metabolism of inositol phosphates in striatal neurons. *Mol Pharmacol* **32**: 364–368, 1987.
10. Nicoletti F, Iadarola MJ, Wroblewski JT and Costa E, Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: Developmental changes and interaction with alpha-1-adrenoreceptors. *Proc Natl Acad Sci USA* **83**: 1931–1935, 1986.
11. Berridge R, Downes CP and Hanley MR, Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem J* **206**: 587–595, 1982.
12. Fisher SK and Bartus RT, Regional differences in the coupling of muscarinic receptors to inositol phospholipid hydrolysis in guinea pig brain. *J Neurochem* **45**: 1085–1095, 1985.
13. Minneman KP and Johnson RD, Characterization of

alpha-1 adrenergic receptors linked to [^{3}H]inositol metabolism in rat cerebral cortex. *J Pharmacol Exp Ther* **230**: 317–323, 1984.
14. Johnson RD and Minneman KP, Differentiation of alpha-1-adrenergic receptors linked to phosphatidylinositol turnover and cyclic AMP accumulation in rat brain. *Mol Pharmacol* **31**: 239–246, 1987.
15. Fisher SK and Snider RM, Differential receptor occupancy requirements for muscarinic cholinergic stimulation of inositol lipid hydrolysis in brain and in neuroblastomas. *Mol Pharmacol* **32**: 81–90, 1987.
16. Jope RS, Casebolt TL and Johnson GVW, Modulation of carbachol-stimulated inositol phospholipid hydrolysis in rat cerebral cortex. *Neurochem Res* **12**: 693–700, 1987.
17. Gonzales RA and Crews FT, Characterization of the cholinergic stimulation of phosphoinositide hydrolysis in rat brain slices. *J Neurosci* **4**: 3120–3127, 1984.
18. Brown E, Kendall DA and Nahorski SR, Inositol phospholipid hydrolysis in rat cerebral cortical slices: I. Receptor characterization. *J Neurochem* **42**: 1379–1387, 1984.
19. Gonzales RA and Crews FT, Cholinergic- and adrenergic-stimulated inositide hydrolysis in brain: Interactions, regional distribution, and coupling mechanisms. *J Neurochem* **45**: 1076–1084, 1985.