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Seizures Increase Acetylcholine and Choline Concentrations in Rat Brain Regions

Richard S. Jope[1] and Xin Gu[1]

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Seizures induced by three convulsant treatments produced differential effects on the concentration of acetylcholine in rat brain. Status epilepticus induced by (i) coadministration of lithium and pilocarpine caused massive increases in the concentration of acetylcholine in the cerebral cortex and hippocampus, (ii) a high dose of pilocarpine did not cause an increase of acetylcholine, and (iii) kainate increased acetylcholine, but the magnitude was lower than with the lithium/pilocarpine model. The finding that the acetylcholine concentration increases in two models of status epilepticus in the cortex and hippocampus is in direct contrast with many *in vitro* reports in which excessive stimulation causes depletion of acetylcholine. The concentration of choline increased during seizures with all three models. This is likely to be due to calcium- and agonist-induced activation of phospholipase C and/or D activity causing cleavage of choline-containing lipids. The excessive acetylcholine present during status epilepticus induced by lithium and pilocarpine was responsive to pharmacological manipulation. Atropine tended to decrease acetylcholine, similar to its effects in controls. The N-methyl-D-aspartate (NMDA) receptor antagonist, MK-801, reduced the excessive concentration of acetylcholine, especially in the cortex. Inhibition of choline uptake by hemicholinium-3 (HC-3) administered icv reduced the acetylcholine concentration in controls and when given to rats during status epilepticus. These results demonstrate that the rat brain concentrations of acetylcholine and choline can increase during status epilepticus. The accumulated acetylcholine was not in a static, inactive compartment, but was actively turning-over and was responsive to drug treatments. Excessive concentrations of acetylcholine and/or choline may play a role in seizure maintenance and in the neuronal damage and lethality associated with status epilepticus.

KEY WORDS: Acetylcholine; lithium; kainate; pilocarpine; status epilepticus.

INTRODUCTION

In a previous study, we reported that acetylcholine concentrations in rat cerebral cortex and hippocampus increased to unprecedentedly high levels (451% and 304% of controls, respectively) during status epilepticus which had been induced by administration of lithium and pilocarpine (1). This model of seizures involves administration of a normally nonconvulsive dose of pilocarpine to rats that were pretreated with lithium (2-4). The time of onset and severity of seizures are very reproducible and the generalized, convulsive status epilepticus that is produced always continues unabated for several hours until death ensues. The initiation of the seizures is due to activation of muscarinic cholinergic receptors, but the mechanisms accounting for the sustained maintenance of status epilepticus are not known, although there is evidence for the involvement of excitatory amino acids (5). The large increase in acetylcholine during status epilepticus was surprising because the concentration of acetylcholine is normally tightly regulated, and stimulation usually tends to deplete acetylcholine (6). The concen-

[1] Department of Psychiatry and Behavioral Neurobiology, Sparks Center, Rm. 910, University of Alabama at Birmingham, Birmingham, Alabama 35294

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tration of choline also rose excessively during the seizures (1).

In the present study, we examined whether the large increases in acetylcholine and choline concentrations were unique to the lithium/pilocarpine model of status epilepticus and if anticonvulsants or other drugs modified this response. To examine if the cholinergic trigger mechanism was a requisite for these effects, and to examine if lithium treatment influenced the accumulation of acetylcholine or choline, we compared concentration changes induced by lithium and pilocarpine to those induced by a high, convulsive dose of pilocarpine (in the absence of lithium), and examined the effects of a convulsive dose of kainate (7) in the presence and absence of lithium pretreatment. Further experiments were carried out to determine if the acetylcholine that was present in excessively high concentrations during status epilepticus induced by lithium and pilocarpine was in a dynamic, actively turning-over pool by examining the effects of a number of drug treatments.

EXPERIMENTAL PROCEDURE

Animal Treatments. Seizures were induced in adult, male Sprague Dawley rats (200-240 gm) by the following drug treatments: (i) LiCl (3 mmole/kg; ip) was administered approximately 20 hr prior to pilocarpine (30 mg/kg; sc); (ii) pilocarpine (380 mg/kg; sc) was administered alone; (iii) kainate (10 mg/kg; sc) was administered to lithium-naive or lithium-treated (as above) rats. Methylatropine (5 mg/kg) was given with pilocarpine to block peripheral cholinergic stimulation, but it did not alter the convulsant effects of pilocarpine.

Some rats were given the following drugs: MK-801 (4 mg/kg; ip), atropine (5 mg/kg; ip), verapamil (25 mg/kg; ip), each administered in 0.1-0.2 ml saline, or diazepam (5 mg/kg; ip). For administration of HC-3, rats were anesthetized with halothane, an incision was made in the scalp, a cannula was stereotaxically placed above the ventricle, and HC-3 in saline (1 μl) was infused. One minute later the cannula was removed, the hole in the skull was filled with bone wax, and the scalp was sutured. The entire process took less than 10 min.

Acetylcholine and Choline. All rats were killed by microwave irradiation focussed on the head (3.5 kW; Gerling Labs, Modesto, CA). The indicated brain regions were removed, weighed, and homogenized in 2 ml 15% 1 N formic acid/85% acetone containing 5 nmole of deuterated acetylcholine and choline as internal standards. Samples were extracted as described (8) and acetylcholine and choline were quantitated by the method of Jenden et al. (9) using gas chromatography and mass spectrometry (GCMS). Statistical significance was evaluated using ANOVA.

Materials. Lithium chloride, pilocarpine hydrochloride, methylatropine, atropine sulfate, verapamil hydrochloride and kainic acid were from Sigma Chemical Co. (St. Louis, MO). MK-801 was a generous gift from Dr. Richard Ransom (Merck Sharp & Dohme Research Laboratories). Diazepam (sterile injectable Valium) was from Roche. Hemicholinium-3 was from Aldrich Chemical Co. (Milwaukee, WI).

RESULTS

Seizure Models. Three different seizure models were used, each of which has been described in detail previously (3,4,10,11). First, administration of a normally nonconvulsive dose of pilocarpine (30 mg/kg) to rats pretreated with LiCl (3 mmole/kg) caused generalized paroxysmal spikes and spike trains after about 15 min which developed into convulsive status epilepticus approximately 20 min after pilocarpine administration and continued unabated for several hours (3,4). Second, administration of a high dose of pilocarpine (380 mg/kg), without utilization of LiCl, caused seizures with a similar initial time course to the lithium plus pilocarpine model, but status epilepticus did not develop until 40 to 60 min after pilocarpine (5). Third, administration of kainate (10 mg/kg) also caused seizures, but the time course was very different. Kainate induced paroxysmal spikes and spike trains, which appeared intermittently between 10 and 96 min after kainate administration, followed by status epilepticus (4). Treatment with LiCl did not significantly alter the latency to any of the stages of kainate-induced seizures or the severity (11).

Effects of Seizures on Acetylcholine. As reported previously in greater detail (1), the concentration of acetylcholine was extremely elevated in the cortex and hippocampus, but unchanged in the striatum, during seizures induced by administration of pilocarpine to lithium-treated rats (Figure 1). The acetylcholine reached peak concentrations that were 380% and 251% of control values in the cortex and hippocampus, respectively, 150 min after pilocarpine administration. These values are similar to those that we published previously, although in the previous work a more extended time course was followed which also revealed initial (15 min) and later (3.5 hr) decreased acetylcholine concentrations (1).

The effects of seizures induced by a high dose of pilocarpine on acetylcholine concentrations were very different from those of the lithium/pilocarpine model. The high dose of pilocarpine reduced the concentration of acetylcholine in all three regions 1 hr after treatment (it should be noted that the lithium/pilocarpine model also decreases the acetylcholine concentration at an early, but different [15 min], time period). This decrease was followed by a return to control values in the cortex, an increase to slightly above (27%) controls in the hippocampus, and a return to control values followed by a secondary decrease in the striatum.

Seizures induced by kainate caused a relatively slow rise in the concentration of acetylcholine in the cortex and hippocampus, with significant increases occurring only after status epilepticus was obtained. Maximal in-

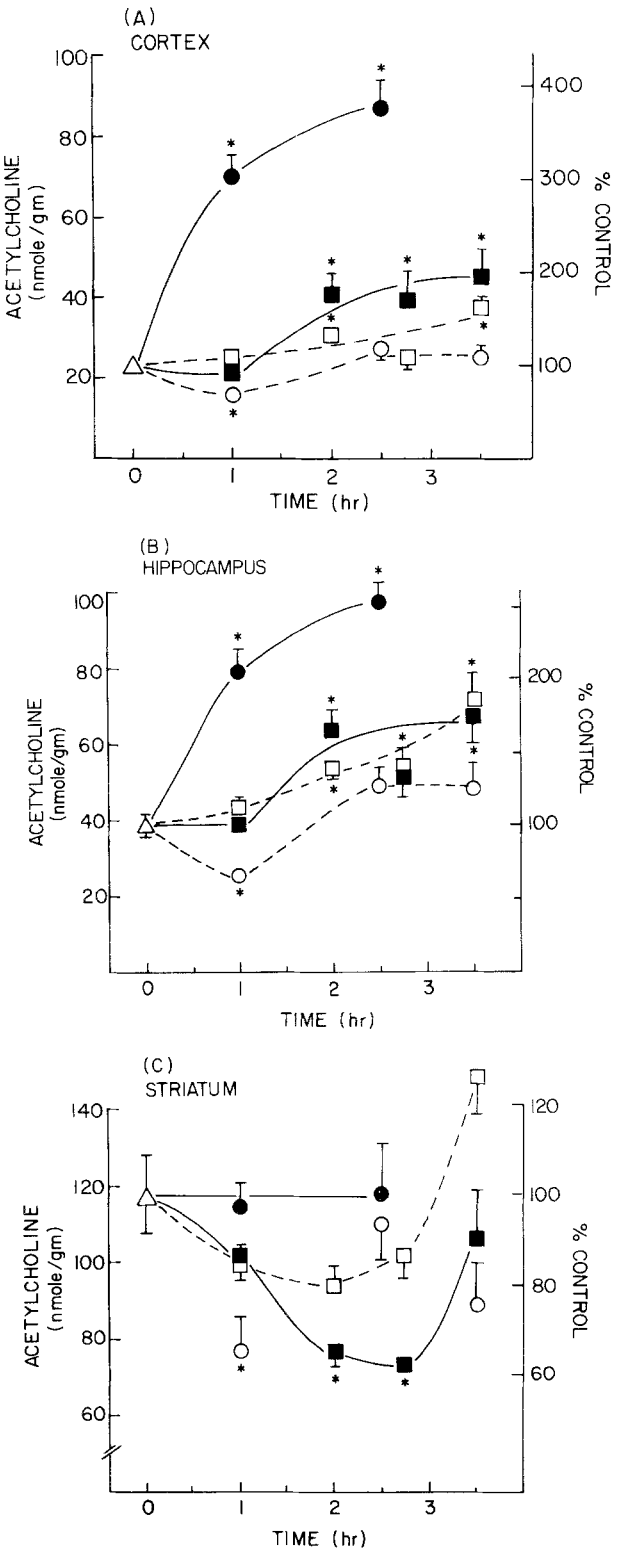

Fig. 1. Acetylcholine concentrations in (A) cortex, (B) hippocampus, (C) striatum of rats treated with epileptogenic agents. Rats were treated with the following drugs: (●) LiCl (3 mmol/kg; i.p.) 20 hr prior to pilocarpine (30 mg/kg; s.c.); (○) pilocarpine (380 mg/kg; s.c.); (□) kainate (10 mg/kg; s.c.); (■) LiCl (3 mmol/kg; i.p.) 20 hr prior to kainate (10 mg/kg; s.c.). Zero times indicate the time at which pilocarpine or kainate was administered. Rats were killed by focussed-beam microwave irradiation, the three regions were isolated and weighed and the acetylcholine concentrations were measured by GCMS as described in the Methods. Values are means ± SEM of 4-8 animals. *$p<0.05$.

creases were observed at the longest time point tested, which were 195% and 185% of controls in the cortex and hippocampus, respectively. In the striatum, kainate caused a decrease in the concentration of acetylcholine and it did not rise to near control values until 3.5 hr after kainate administration.

Pretreatment with lithium did not cause a large change in the response of the acetylcholine concentrations to kainate, as it did with pilocarpine. There was a tendency for lithium pretreatment to enhance the accumulation of acetylcholine in the cortex and the depletion of acetylcholine in the striatum.

Effects of Seizures on Choline. Each of the treatments that were tested increased the concentration of choline in all three brain regions that were examined, but the increase occurred at varying rates and to varying degrees dependent upon the treatment.

Treatment with lithium and pilocarpine (30 mg/kg) and the attendant seizures caused the most pronounced elevations of choline in each of the regions (Figure 2). The concentration of choline rose rapidly to at least double control values 1 hr after pilocarpine administration to lithium-treated rats. In the cortex and hippocampus the choline concentration continued to increase over time, reaching values over 3-fold those of controls. These results are consistent with the more extensive time course that we published previously (1). Treatment with either lithium or pilocarpine alone did not alter acetylcholine or choline concentrations under these conditions (1).

Sixty minutes after administration of a convulsive dose of pilocarpine (380 mg/kg) the choline concentration was unaltered in the cortex and hippocampus, but was depressed in the striatum. Subsequently, there was a rise over time in the concentration of choline in all three regions. Three and one-half hours after pilocarpine administration, the concentration of choline was increased by approximately 2-fold or greater in all regions, with the largest increase occurring in the hippocampus, which was approximately 300% of control values.

The effects of kainate and of kainate plus lithium on the concentrations of choline were similar in each of the regions, but the response in the hippocampus was most pronounced. In each region the concentration of choline increased after each treatment and reached a

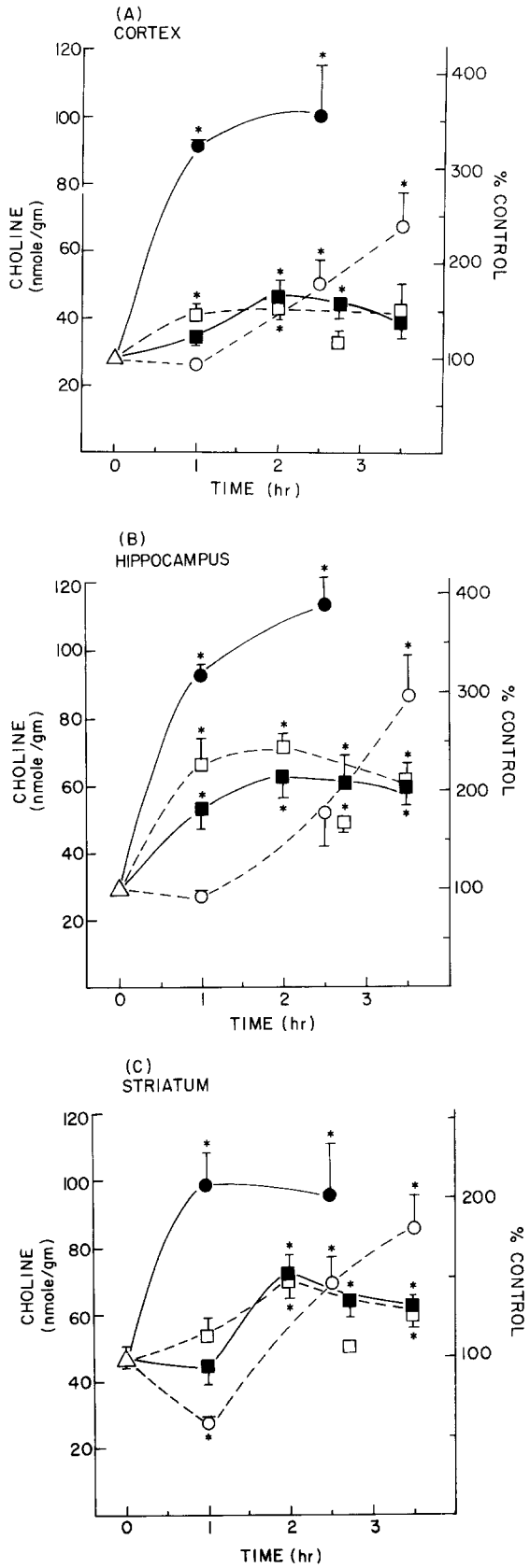

Fig. 2. Choline concentrations in (A) cortex, (B) hippocampus, and (C) striatum of rats treated with epileptogenic agents. Methods and treatments are described in the legend to Figure 1. Symbol representations are: (●) pilocarpine (30 mg/kg) administered to lithium-treated rats; (○) pilocarpine (380 mg/kg); (■) kainate (10 mg/kg) administered to lithium-treated rats; (□) kainate (10 mg/kg). Values are means ± SEM of 4-8 animals. *$p<0.05$.

maximum value 2 hr after administration of kainate. The maximum value was over 200% of controls in the hippocampus and approximately 150% of controls in the cortex and striatum. In each region there was a tendency for the choline to be elevated less in the lithium-pretreated rats than in those treated only with kainate at 1 hr, but not at other times.

Effects of Drugs on Seizure-Induced Elevations of Acetylcholine and Choline. We reported previously that the acetylcholine which accumulated during lithium/pilocarpine-induced status epilepticus was in a bound, calcium-dependent releasable compartment indistinguishable from that in normal rat brain (1). In an attempt to determine if the excessive acetylcholine was active in vivo and was responsive to pharmacological manipulation, a number of drugs were tested in conjunction with lithium/pilocarpine-induced status epilepticus for effects on the accumulation of acetylcholine. Similarly, we examined whether these agents altered the release of choline during seizures. In these experiments, drugs were administered 60 min after pilocarpine and their effects during the subsequent 90 min were measured. Therefore, values were compared to those in lithium/pilocarpine rats measured 150 min after pilocarpine treatment, the time at which the acetylcholine concentration was maximal.

Administration of atropine to control rats caused depletion of acetylcholine (1). In seizing rats atropine had a similar effect, as it substantially reduced the seizure-induced increase of acetylcholine in the cortex and hippocampus, and reduced it in the striatum below that of untreated controls (Figure 3). It should be noted that atropine administered during status epilepticus did not alter the seizures or the EEG (3).

The most effective drug that we have found for reducing seizures when administered during (rather than prior to) status epilepticus is the NMDA antagonist MK-801 (5). Administration of MK-801 during status epilepticus significantly reduced the acetylcholine concentration in the cortex and striatum to below that obtained at 60 min, and blocked further increases in the hippocampus. EEG recordings have shown that the anticonvulsant effect of MK-801 occurs more rapidly in the

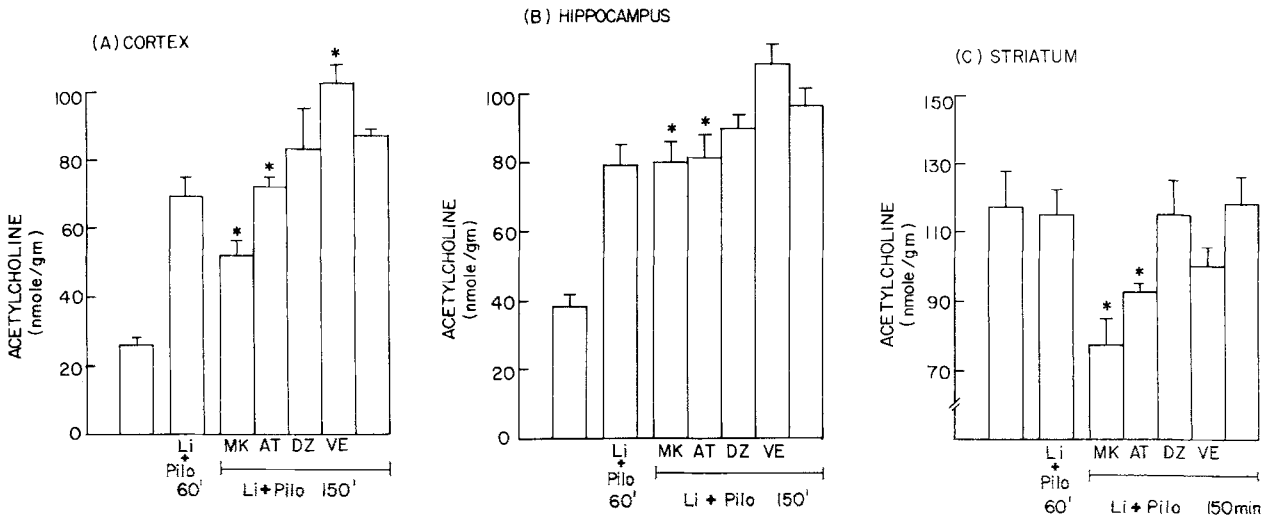

Fig. 3. Acetylcholine concentrations in (A) cortex, (B) hippocampus, and (C) striatum. Acetylcholine was measured by GCMS following microwave irradiation. Treatments and symbols are as follows from left to right: control, lithium (3 mmole/kg; ip; 20 hr prior) and pilocarpine (30 mg/kg; sc; 60 min prior to sacrifice). The remaining rats were given lithium and pilocarpine and 60 min after pilocarpine the following drugs were administered followed by sacrifice 150 min after pilocarpine: MK-801 (4 mg/kg; ip), atropine (5 mg/kg; ip), diazepam (5 mg/kg; ip), verapamil (25 mg/kg; ip), and no additional drug. Values are means ± SEM (n = 3-6). *$p<0.05$ compared with lithium and pilocarpine (150 min).

cortex than in the hippocampus (5). MK-801 did not alter the concentration of acetylcholine in otherwise untreated rats except for an increase in the striatum (possibly due to blockade of NMDA-induced release of acetylcholine (12)) and administration of MK-801 prior to pilocarpine both blocked the development of status epilepticus and the increased concentration of acetylcholine (data not shown).

Administration of the calcium antagonist verapamil or the benzodiazepine, diazepam, had little effect on the concentration of acetylcholine and also did not alter the EEG (data not shown).

The concentration of choline also increased during lithium/pilocarpine-induced status epilepticus. The increased choline was most responsive to drug treatments in the cortex and hippocampus, but the striatum was less affected. Atropine administered during seizures elevated choline whereas MK-801 tended to reduce choline (Figure 4). Diazepam did not alter choline and verapamil tended to elevate the concentration of choline. Pretreatment with MK-801 blocked seizures and the increase in choline (data not shown).

Hemicholinium-3 (HC-3), an inhibitor of the high affinity choline transport system which regulates the synthesis of acetylcholine, was utilized to determine if the acetylcholine which accumulated during seizures continued to be utilized, to estimate if the acetylcholine turnover rate was altered during status epilepticus, and to determine if the high affinity choline transport system continued to play a limiting role in the synthesis of acetylcholine.

Figure 5 shows in control rats the dose-dependency and time-course of the reduction of acetylcholine concentrations induced by HC-3. Considering these results, an intermediate dose of HC-3 (10 μg) was utilized in treated rats and a one hour treatment period was employed. For these experiments, pilocarpine was administered to lithium treated rats, HC-3 was administered after one hour, and acetylcholine and choline concentrations were measured one hour later and compared with those in sham-operated, seizing controls. In the cortex and hippocampus, seizures resulted in increased concentrations of acetylcholine, whereas there was no significant change in the striatum, as reported previously (1). Administration of HC-3 to rats during status epilepticus induced significant declines in the concentrations of acetylcholine in all three regions, and these reductions are compared in Figure 5 to those measured in control rats using the same dose of HC-3 and time period. It is evident that the high affinity choline transport system continued to play an obligatory role in the synthesis of acetylcholine during status epilepticus. Furthermore, the decline of acetylcholine synthesis after HC-3 provides a relative comparison of the turnover rate of acetylcholine in each brain region during status epilepticus compared with controls. It is evident that the acetylcholine that

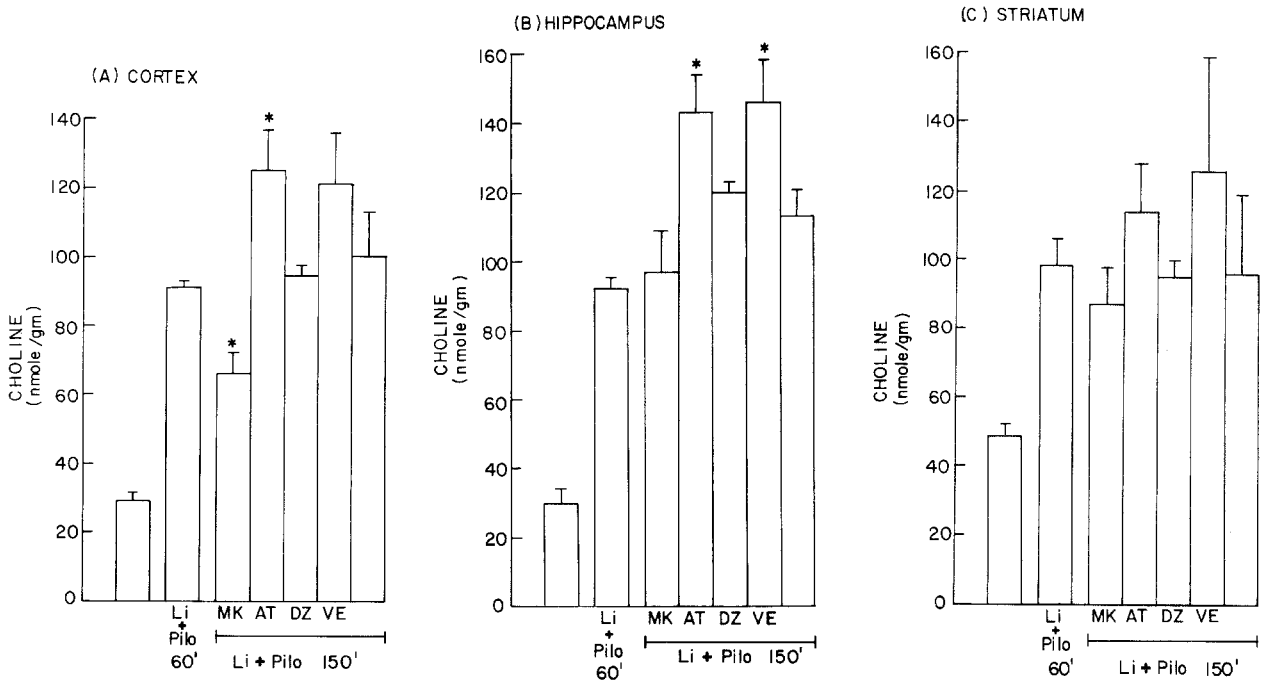

Fig. 4. Choline concentrations in (A) cortex, (B) hippocampus, and (C) striatum. Treatments and symbols are as described in the legend to Figure 3.

accumulated during status epilepticus continued to turn over, and that the turnover rate was apparently increased in the cortex, unchanged in the hippocampus and possibly reduced in the striatum, although this method provides only a crude estimation of the acetylcholine turnover rate.

DISCUSSION

Coadministration of lithium and pilocarpine causes generalized convulsive status epilepticus in rats which continues unabated for several hours. The reproducibility of the responses to this treatment make this a very useful model with which to investigate various facets of seizures (4). Although excessive stimulation might be predicted to deplete acetylcholine, on the contrary acetylcholine concentrations reach very high levels in both the cerebral cortex and hippocampus during these seizures. Administration of a convulsant dose of kainate which also causes status epilepticus also resulted in large elevations of the concentration of acetylcholine in the cortex and hippocampus. Although the increases were not as great as those observed with the lithium/pilocarpine model, this comparison should not diminish the surprising fact that acetylcholine was not depleted, but actually accumulated, during status epilepticus induced by this excitatory amino acid agonist. As with the lithium/pilocarpine model, acetylcholine in the striatum responded differently from the other two regions, as kainate treatment caused depletion, not elevation, of striatal acetylcholine. In contrast to the other two models, seizures produced by a high dose of pilocarpine (without lithium pretreatment) did not cause large increases in the concentration of acetylcholine. These studies show that there is the potential for acetylcholine to reach high concentrations for long periods of time during status epilepticus, but that the magnitude of the change must be regulated by as yet unidentified differences among the models of status epilepticus in the responses of cholinergic neurons.

Severe stimulation of neuronal activity might be predicted from in vitro studies to result in depletion of acetylcholine (6). However, on the contrary, in both the cerebral cortex and hippocampus there was little evidence of depletion but a clear accumulation of acetylcholine in two models of status epilepticus. The exaggerated response with the lithium/pilocarpine model appears not to be due to the presence of lithium during seizure activity, since lithium treatment did not greatly magnify the effects of kainate on acetylcholine. The differences in the degree of elevation of acetylcholine among

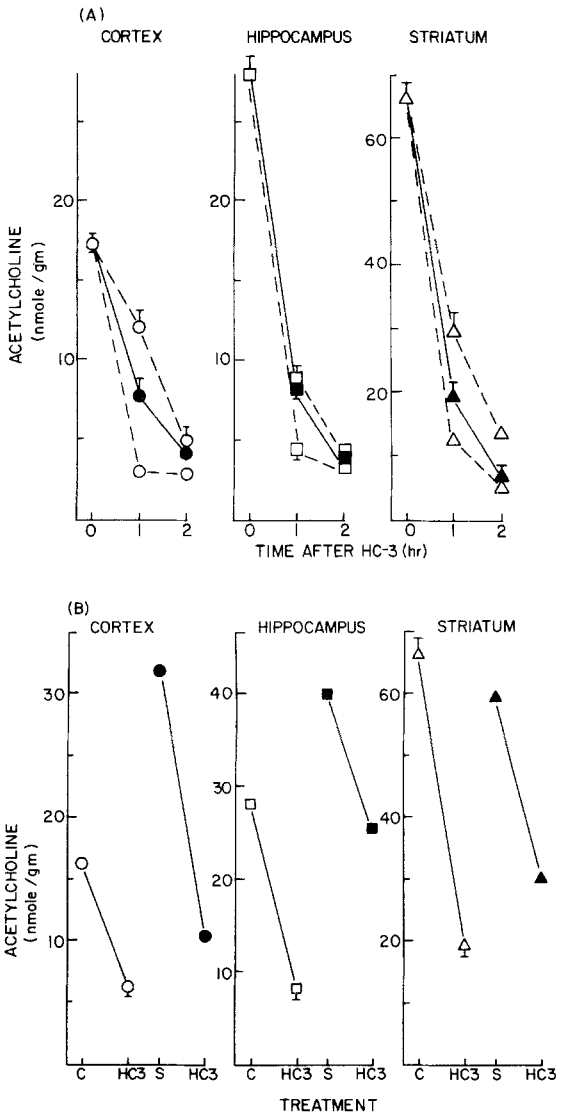

Fig. 5. Acetylcholine concentrations in rat brain regions after HC-3 treatment. (A) Control rats were given icv HC-3 (1[top open symbol], 10 [filled symbol], or 20 μg [bottom open symbol]) one or two hour prior to sacrifice by microwave irradiation. The concentration of acetylcholine was measured by GCMS as described in the Methods. Solid lines and filled symbols indicate the dose (10 μg) used in later experiments with seizing rats. Means ± SEM (n = 4-6). (B) Open symbols indicate values obtained in control rats and controls given HC-3 (10 μg; icv) one hour prior to sacrifice. Closed symbols indicate values in rats pretreated with LiCl (3 mmole/kg; ip; 20 hr prior) and pilocarpine (30 mg/kg; sc). One hr after pilocarpine (during seizures) HC-3 (10 μg) was infused in 1 μl (rats not receiving HC-3 were sham operated and all rats were anesthetized for equivalent periods of time), and one hour elapsed before sacrifice by microwave irradiation. As determined by EEG in rats run in parallel (electrodes prevent sacrifice by microwave irradiation) and behaviorally in rats to be used for biochemical measurements, this procedure with halothane anesthesia did not prevent continuation of status epilepticus, however it did lessen the degree of the increase in acetylcholine. The figure shows values in untreated controls (C), controls given HC-3 (HC3) one hour previously, and rats given lithium and pilocarpine (2 hour prior) with either sham operated (S) or HC-3 (HC3) one hour prior to sacrifice. The lines indicate the decrease in acetylcholine concentrations due to administration of HC-3. Means ± SEM (n = 4).

the models may be an indication of the differences in severity and continuity of seizure activity, as the lithium/pilocarpine model enhances these aspects more reliably than either of the other two models tested. With all of these models, the reason for the differential response of acetylcholine in the striatum remains unanswered, but it may be due to the unique cholinergic structures in this region in comparison with the other two that were studied. Nonetheless, the observation that the acetylcholine concentration increases in the cortex and hippocampus during severe seizure activity demonstrates that the cholinergic system in vivo responds differently from in vitro preparations in which excessive stimulation often results in large depletions of acetylcholine.

Interest in agents which modulate choline concentrations has increased in recent years since it was discovered that much of the free choline that is generated is due to activation of receptor-coupled hydrolysis of phosphatidylcholine by phospholipase C or D (13). Thus, it is now realized that choline is not generated by nonspecific membrane degradation, but by a highly controlled, second messenger linked complex. The concentration of choline tended to increase in all regions during seizures irregardless of the convulsive agent, but the increases were greatest with the lithium/pilocarpine model. Notably, choline in the hippocampus tended to increase to a greater extent than it did in the other two regions. Compared with the lithium/pilocarpine model, there was a slower rate of rise of choline following administration of a high dose of pilocarpine. This lag is probably accounted for by the longer latency to status epilepticus (approximately 47 min compared with 20 min) (5), but even during status epilepticus the elevation of choline was greater in the lithium/pilocarpine model. Kainate also caused accumulation of choline, especially in the hippocampus. As with acetylcholine, coadministration of lithium with kainate did not cause major alterations in the release of choline. A part of the increased choline during seizures may be generated by activation of phospholipase C and/or D by accumulation of intracellular calcium (13). However, selective agonist-dependent activation is also suggested by the differential degrees of change observed among these models.

The effects of administration of atropine or MK-801 during status epilepticus on the concentration of acetylcholine support the conclusion that despite the extraordinarily high concentration of acetylcholine, it continues to exist in a functional intracellular compartment (1). Thus, both atropine and MK-801 blocked further accumulation of acetylcholine or led to a significant decrease. Atropine induces release and depletion of acetylcholine in control rats and these results indicate that

it has a similar effect on the acetylcholine accumulated during status epilepticus. MK-801 did not alter the acetylcholine concentration in control rats, suggesting that the accumulation during seizures involves activation of NMDA receptors. It is likely that this effect of MK-801 to reduce acetylcholine concentrations is due to its anticonvulsive effects, since the effects on the EEG and on acetylcholine were more evident in the cortex than the hippocampus and since activation of NMDA receptors is reported to increase, rather than decrease, the release of acetylcholine (12). We have shown previously in studies with MK-801 that activation of NMDA receptors plays a major role in the maintenance of status epilepticus after induction by administration of lithium plus pilocarpine (5).

The increased concentration of choline during status epilepticus was also modulated by additional drug treatments. Atropine increased the choline concentration, likely as a result of atropine-stimulated acetylcholine release and its subsequent hydrolysis by acetylcholinesterase. MK-801 tended to reduce the choline concentration, with the most pronounced effect occurring in the cortex. Considering the previous findings with MK-801 discussed above, this is evidently associated with the anticonvulsant response to MK-801. Verapamil tended to enhance the elevation of choline, but the mechanism for this response is not obvious since verapamil has no effect on the EEG in this model (unpublished observations).

Administration of HC-3, which blocks the regulatory high affinity choline transport carrier, caused reductions in the acetylcholine concentrations in all three brain regions. These decreases were remarkably similar to the HC-3-induced reductions measured in control rats. This demonstrates that, although the concentration of acetylcholine is much higher in rats undergoing seizures than in controls, it continues to turn over. Furthermore, these data indicate that the activity of the high affinity choline transport system remains obligatory for acetylcholine synthesis even during seizures when both the acetylcholine and choline concentrations are elevated. The question remains as to why acetylcholine accumulates to such a large degree during seizures. Since incubation of brain slices with lithium and pilocarpine does not cause accumulation of acetylcholine (or choline) (unpublished observations), the regulatory parameters are difficult to identify. The breakdown of normal regulatory mechanisms which usually maintain the concentrations of acetylcholine and choline may play a role in the long-lasting and degenerative effects of status epilepticus.

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