Role of AMPA and GluR5 kainate receptors in the development and expression of amygdala kindling in the mouse

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Abstract

The role of AMPA and GluR5-containing kainate receptors in the development and expression of amygdala kindling was examined using the selective 2,3-benzodiazepine AMPA receptor antagonist GYKI 52466 [(1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine] and the decahydroisoquinoline mixed AMPA receptor and GluR5 kainate receptor antagonist LY293558 {[(3S,4aR,6R,8aR)-6-[2-(1(2H)-tetrazole-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid]}. Administration of GYKI 52466 (5–40 mg/kg, intraperitoneally) and LY293558 (10–40 mg/kg, intraperitoneally) prior to daily kindling stimulation in mice produced a dose-dependent suppression of the rate of development of behavioral kindled seizure activity and reduced the duration of the stimulation-induced electrographic afterdischarge. In drug-free stimulation sessions after the initial drug-treatment sessions, there was an acceleration in the rate of kindling development compared with the rate during the preceding drug-administration period; the “rebound” rate was also greater than the kindling rate in saline-treated control animals. In fully kindled animals, both GYKI 52466 and LY293558 produced a dose-dependent suppression of evoked seizures (ED50, 19.3 and 16.7 mg/kg, respectively). Although AMPA receptors appear to be critical to the expression of kindled seizures, since kindling development progressed despite the suppression of behavioral seizure activity, AMPA receptors are less important to the kindling process. LY293558 was modestly less effective at suppressing behavioral seizures during kindling and was not superior to GYKI 52466 in retarding the overall extent of kindling development, indicating that GluR5 kainate receptors do not contribute to epileptogenesis in this model. Published by Elsevier Science Ltd.

Keywords: Kindling; Amygdala; Seizure; AMPA receptor; Kainate receptor

1. Introduction

The kindling model has been widely used to assess the role of excitatory and inhibitory synaptic receptor systems in the development and expression of limbic epilepsy (Löschner, 1998). In amygdala kindling, a mild focal electrical stimulus is applied unilaterally to the amygdala on a daily basis. With time, the initially behaviorally innocuous electrical stimulus elicits increasingly more robust limbic seizure-like activity (Goddard et al., 1969). It is well recognized that N-methyl-d-aspartate (NMDA) receptor antagonists retard the development of kindling, but are less active in blocking the expression of fully kindled seizures (Holmes et al., 1990; Croucher and Bradford, 1990; Katayama et al., 1990; Sato et al., 1998; Morimoto et al., 1991; Namba et al., 1994). In contrast, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor antagonists are highly effective in protecting against fully kindled seizures. One early report observed that AMPA receptor antagonists do not interfere with kindling development (Dürmüller et al., 1994). However, other investigators have concluded that AMPA receptor antagonists do indeed have antiepileptogenic activity (Namba et al., 1994; Kodama et al., 1999). Therefore, the role of AMPA receptors in kindling epileptogenesis is still incompletely defined.

The recent demonstration that 2,3-benzodiazepines such as GYKI 52466 are markedly more potent as antagonists of AMPA receptors containing the GluR1–4 sub-
units than of kainate receptors composed of the GluR5, GluR6 and KA-2 subunits (Paternain et al., 1995), and the availability of decahydroisoquinolines such as LY293558 with variable activity at AMPA receptors that are also capable of selectively blocking GluR5 kainate receptors (Ornstein et al., 1993; Bleakman et al., 1996; Clarke et al., 1997; Simmons et al., 1998), have made it possible to discriminate pharmacologically between AMPA and GluR5 kainate receptors. Using these antagonists, we recently demonstrated that GluR5 kainate receptors participate in synaptic transmission in the amygdala (Li and Rogawski, 1998). Our observations raise the possibility that GluR5 kainate receptors could play a role in amygdala kindling. Therefore, in the present study, we sought to determine the importance of GluR5 kainate receptors in the development and expression of amygdala kindling. In addition, in view of the controversy noted above, we endeavored to re-evaluate the role of AMPA receptors in kindling development. Our results indicate that AMPA receptors are crucial to the expression but not the development of behavioral kindled seizures, and that GluR5 kainate receptors are also not required for epileptogenesis in this model.

2. Methods

2.1. Animals

Male National Institutes of Health (NIH) Swiss mice weighing approximately 30 to 35 g were obtained from the NIH Animal Supply Program. The mice were housed in an environmentally controlled animal facility under a 12 h/12 h light/dark cycle and allowed free access to food and water, except during the experimental sessions. All procedures were performed in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the NIH Animal Use Committee.

2.2. Electrode implantation

Animals were anesthetized by intraperitoneal (i.p.) injection of a mixture of ketamine (100 mg/kg) and xylazine (20 mg/kg). A twisted bipolar stainless steel wire electrode (model MS303/1; Plastic One, Roanoke, VA) was stereotaxically implanted in the right amygdala com-plex (1.3 mm posterior and 3.0 mm lateral to bregma, and 4.6 mm below the dorsal surface of the skull) and anchored with dental acrylic to three jeweler’s screws placed in the skull. A period of 7 to 10 days was allowed for recovery.

2.3. Kindling procedure

The afterdischarge threshold was determined by stimulating at 5 min intervals beginning at an intensity of 75 μA and increasing in steps of 50 μA until an afterdischarge of at least 5 s was obtained. The stimulation paradigm consisted of 1 ms duration, bipolar, square current pulses delivered at 60 Hz for 1 s. Stimulation on subsequent days used a stimulation intensity 125% of the threshold value. Seizure activity following each stimulation was rated according to the criterion of Racine (1972) as modified for the mouse: stage 0, no response or behavior arrest; stage 1, chewing or head nodding; stage 2: chewing and head nodding; stage 3: forelimb clonus; stage 4: bilateral forelimb clonus and rearing; stage 5: falling. The afterdischarge was recorded from the amygdala electrode with a Grass CP511 AC electroencephalogram preamplifier (Astro-Med, West Warwick, RI) and stored in digital form using Axotape 2.02 (Axon Instruments, Foster City, CA). Afterdischarge duration was the total duration of amygdala electroencephalographic spike activity (amplitude >2 × baseline) occurring in a rhythmic pattern at a frequency >1 Hz. The afterdischarge amplitude often exhibited waxing and waning; when this occurred, the duration was considered as the time to the end of the last spike discharge in a sequence consisting of more than three spikes. Stimulation was continued on a daily basis each afternoon between 13:30 and 16:00 h for five days per week.

2.4. Kindling development and expression studies

The day of afterdischarge threshold determination was considered day 1 of kindling. In the kindling development study, animals received an intraperitoneal injection of vehicle or antagonist 15 min prior to stimulation on days 3 to 14. Daily kindling stimulation was continued until stage 5 seizures were elicited on three consecutive days. Drug-treated animals exhibited reduced locomotor activity and incoordination, and at the highest doses were largely immobile.

In a second group of animals, kindling stimulation was delivered daily (five days per week) without vehicle or drug treatment until stage 5 seizures were elicited on three consecutive days. On the first and third day after the criterion was met, animals received an intraperitoneal drug injection 15 min prior to stimulation. The stimulations on the two days preceding the drug administration were taken as control for purposes of determination of afterdischarge inhibition.

2.5. Data analysis

Group data are expressed as the mean±standard error of the mean (SEM). Changes in seizure stage were compared with the Kruskal–Wallis test, followed by the Mann–Whitney U-test. Slopes were compared by confidence limits. Comparisons between means of the afterdischarge duration and number of stimulation sessions were made with one-way analysis of variance, followed...
Fig. 1. Effect of GYKI 52466 on kindling development. Mice received an intraperitoneal injection of saline or the indicated dose of GYKI 52466 15 min prior to kindling stimulation on days 3 through 14. Each data point represents the mean of the behavioral seizure stage of seven or eight animals. Small filled circles indicate that the mean value is significantly different from that in saline (P<0.05).

Fig. 2. Effect of LY293558 on kindling development. Mice received an intraperitoneal injection of saline or the indicated dose of LY293558 15 min prior to kindling stimulation on days 3 through 14. Each data point represents the mean of the behavioral seizure stage of seven to nine animals. All animals in the 40 mg/kg group achieved stage 5 with stimulation 32 (not shown). Small filled circles indicate that the mean value is significantly different from that in saline (P<0.05).

by unpaired two-tailed Student’s t-test. Comparison of the mean percentage inhibition of seizure stage and afterdischarge duration in fully kindled animals was by Wilcoxin signed ranks test and paired two-tailed Student’s t-test, respectively. ED\textsubscript{50} (estimated dose resulting in 50% inhibition) values were determined by non-linear curve fitting using the Levenberg–Marquardt algorithm to a logistic equation where the maximum inhibition was assumed to be 100%.

Fig. 3. Mean afterdischarge duration in the experiments of Figs. 1 and 2. P values in a one-way analysis of variance for 40 mg/kg GYKI 52466 and 20 and 40 mg/kg LY293558 are <0.001; in other cases P>0.7. Small filled circles indicate that the mean value is significantly different from that in saline (P<0.05). Afterdischarge duration was not measured in the drug-free period (stimulation 15 and greater) when animals exhibited three successive stage 5 seizures.

2.6. Drugs

GYKI 52466 [(1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride] was obtained from Research Biochemicals International (Natick, MA). LY293558 {(3S, 4aR, 6R, 8aR)-6-[2-(1(2)H-tetrazole-5-yl)ethyl][decahydroisoquinoline-3-carboxylic acid)} was a generous gift of Lilly Research Laboratories (Indianapolis, IN).

3. Results

3.1. Kindling development

The development of amygdala-kindled seizures in saline-treated control mice and in mice that had received injections of various doses of GYKI 52466 and
LY293558 prior to stimulations 3 through 14 is shown in Figs. 1 and 2. In the saline-treated control animals there was a progressive increase in seizure stage, with all animals achieving stage 5 after 17 stimulations. The mean afterdischarge duration did not vary significantly during the course of stimulation in saline-treated animals (range, 24–55 s; \( P = 0.20 \)) (Fig. 3). GYKI 52466 produced a dose-dependent suppression of behavioral seizure activity with significant effects at 10, 20 and 40 mg/kg. With 40 mg/kg GYKI 52466, there was nearly complete suppression of seizures during the drug-treatment phase. Table 1 presents the mean number of stimulations to achieve various seizure stages. All doses of GYKI 52466 greater than 5 mg/kg were associated with significant increases in the number of sessions required. As shown in Fig. 3, doses of GYKI 52466 in the range of 5 to 20 mg/kg did not significantly affect the afterdischarge duration. However, the 40 mg/kg dose produced on average a 75% decrease in the afterdischarge duration (\( P < 0.001 \)).

LY293558 produced a similar suppression of behavioral seizure expression at doses above 10 mg/kg, but even at the highest dose (40 mg/kg) the suppression was not complete during the drug-treatment sessions (Fig. 2, Table 1). At 10 mg/kg, LY293558 did not significantly affect kindling development (Fig. 2) or afterdischarge duration (Fig. 3). At the 20 and 40 mg/kg doses, there was an increase in the number of sessions required to achieve stage 5 kindling (Table 1) and, on average, a 45% and 67% decrease, respectively, in afterdischarge duration (\( P < 0.001 \)).

Drug treatment was terminated after stimulation 14 but daily stimulation was continued until all animals exhibited stage 5 seizures on three consecutive days. To compare the progression of kindling in the drug-treatment period with that in the subsequent drug-free period, the rate of kindling in the two phases was determined as described in the caption to Fig. 4. In the saline control group, there was no significant difference in the rate of kindling in the two phases (Fig. 4). However, as shown in Fig. 4, for GYKI 52466 doses of 10 mg/kg and greater and for LY293558 doses of 20 mg/kg and greater, there was a significant decrease in the rate of behavioral kindling progression during the drug-treatment period and a significant increase in rate after the drug-treatment period. This indicates that the drug treatment inhibits the expression of kindled seizures, but that kindling development continues so that there is a substantial rebound in the apparent rate of kindling when the drug is withdrawn.

3.2. Kindling expression

To evaluate the activity of GYKI 52466 and LY293558 in protecting against fully kindled seizures, animals that had been kindled to stage 5 were treated with various doses of the two antagonists prior to stimulation. Both drugs suppressed the expression of behavioral seizures and, as illustrated in the sample records of Fig. 5, inhibited the afterdischarge. The dose-dependent effects of the two drugs on seizure stage and afterdischarge duration are shown in Fig. 6. The estimated ED\(_{50}\) values for suppression of seizure stage with GYKI 52466 and LY293558 are 19.3±1.4 and 16.7±2.4 mg/kg, respectively. The corresponding values using the afterdischarge data are 27.7±5.2 and 19.1±4.0 mg/kg, respectively.

4. Discussion

The ionotropic glutamate receptors — encompassing receptors of the NMDA, AMPA and kainate classes that subserve fast excitatory transmission in the nervous system (Collingridge and Lester, 1989; Dingledine et al., 1999) — are well recognized to play key roles in the development and expression of seizures in various models of epilepsy, including the kindling model (Rogawski, 1995). The involvement of NMDA and AMPA receptors has been investigated extensively; however, the lack
Fig. 4. Comparison of the rate of kindling development during drug-treatment and drug-free periods. Mean seizure stage values with stimulations 1 through 14 or stimulation 14 through stage 5 kindling (Figs. 1 and 2) were fit to the linear function $S_n = Rn + A$, where $S_n$ is the mean seizure stage value for the $n$th stimulation, $R$ is the rate of kindling, and $A$ is set equal to either 0 (stimulations 1 through 14) or $S_{14}$ (stimulations 14 to stage 5 kindling). The bar chart indicates the $R$ values and their associated standard errors. Correlation coefficients ($r$) were generally $>0.95$ except in the case of the fit to stimulations 1 to 14 for 40 mg/kg GYKI 52466 ($r=0.27$) and to stimulations 14 to stage 5 for 5 mg/kg GYKI 52466 ($r=0.62$). * indicates a significant difference ($P<0.05$) from the corresponding control value (0 mg/kg). In all cases where there is a significant difference from control, the $R$ value for stimulations 1 to 14 is significantly less than for stimulations 14 to stage 5.

Fig. 5. Representative traces illustrating inhibition of afterdischarge in two fully kindled mice by GYKI 52466 (A) and LY293558 (B). Traces show depth electroencephalographic recordings from a right-amygdala-stimulating electrode. Horizontal bar at top indicates period of blanking surrounding the 1 s kindling stimulus. In each trace, a slow negative wave following the stimulation was digitally subtracted. Control traces were obtained in the same animals in the absence of drug treatment.

...until recently of suitable pharmacological agonists and antagonists has hampered studies on the role of kainate receptors. While it has now been amply demonstrated that NMDA receptors are critical to the development of kindled seizures, the participation of AMPA receptors in kindling development per se has been a matter of controversy. We sought to address this issue by comparing the rates of kindling in the presence of AMPA receptor blockade with the rate occurring in the absence of drug treatment. Both GYKI 52466 and LY293558 are effective AMPA receptor antagonists that are active in vivo and have anticonvulsant activity in conventional seizure models (Chapman et al., 1991; Ornstein et al., 1993; Donevan and Rogawski, 1993; Yamaguchi et al., 1993; Schoepp et al., 1995). If the rate of kindling is greater after termination of treatment with these drugs than...
Fig. 6. Inhibition of fully kindled seizures and afterdischarge duration by GYKI 52466 and LY293558. Animals that had been stimulated daily until a stage 5 seizure was elicited on three consecutive days received an intraperitoneal injection of vehicle or drug 15 min prior to stimulation on the subsequent first and third days of stimulation. Percentage inhibition of seizure stage was calculated according to $100\times(1-S/S_{c})$, where $S$ is the average seizure stage in the two drug treatment sessions. Percentage inhibition of afterdischarge duration was calculated according to $100\times(1-D/D_{c})$, where $D$ is the average afterdischarge duration in the two drug-treatment sessions and $D_{c}$ is the average control afterdischarge duration for that animal. The overall mean control afterdischarge duration was 43.7±2.2 s. Each bar represents the mean±SEM of data from six to eight animals. *, $P<0.05$.

under control conditions, this would imply that the irreversible process of kindling development continues during the drug-treatment period, but that drug treatment prevents the behavioral manifestations.

Our results clearly demonstrate that kindling development can continue even when behavioral seizures are suppressed by AMPA receptor blockade. Following termination of daily treatment with both GYKI 52466 and LY293558, there was a marked increase in the rate of kindling development as monitored by the expression of kindled seizures (Fig. 4). The rate was not only greater than the rate occurring in the corresponding drug-treatment period, but was also greater than the rate of kindling in control saline-treated animals. These results strongly suggest that the predominant action of GYKI 52466 and LY293558 is to suppress the expression of kindled behavioral seizures and that the drugs have less effect on the process of kindling development. The situation with GYKI 52466 at a dose of 40 mg/kg dramatically illustrates this point. At this dose, seizure expression was nearly entirely eliminated and afterdischarge duration was reduced by an average of 75%. Nevertheless, the rate of kindling occurring after termination of GYKI 52466 treatment was 2.3 times the rate during the corresponding control period. The results with GYKI 52466 at 20 mg/kg are also instructive. At this dose, there was no significant suppression of afterdischarge duration (Fig. 3), indicating that local electrical seizure activity was largely unimpaired. Nevertheless, behavioral seizures during drug treatment were markedly suppressed. When drug treatment was withdrawn, a large increase in seizure stage occurred (stimulation 15; Fig. 1). This further demonstrates how kindling epileptogenesis can progress even though behavioral seizures are suppressed. Indeed, the progress of epileptogenesis seems to be maximized when local seizure activity is unimpaired. This local seizure activity can presumably activate NMDA receptors that are necessary for the kindling process. AMPA receptor blockade may prevent the spread of seizure activity in the brain so that behavioral manifestations are inhibited, but AMPA receptors do not appear to be critical for the kindling process itself.

Inspection of Figs. 1 and 2 does, however, indicate that the stage of kindling achieved after termination of drug treatment is somewhat below that of the control level obtained with a corresponding number of stimulations. Thus, there is some retardation of kindling. This decrease could be due to the diminution in the spread of electrical activity in the brain and the consequent limitation of NMDA receptor activation. However, the alternative possibility that AMPA receptors participate to some extent in the kindling process cannot be entirely eliminated.

In line with the idea that AMPA receptor blockade suppresses behavioral seizure activity during kindling development, we found that GYKI 52466 and LY293558 were highly effective at protecting against fully kindled seizures (Fig. 6). The potencies of both drugs were modestly lower than those required for protection in some acute seizure models (Chapman et al., 1991; Yamaguchi et al., 1993; Ornstein et al., 1993; Schoepp et al., 1995) but within the ranges required for neuroprotection (Gill and Lodge, 1994; Block et al., 1996; O’Neill et al., 1998). The present results further support the potential utility of AMPA receptor antagonists in epilepsy therapy (Rogawski and Donevan, 1999).

An additional aim of the present study was to determine if GluR5 kainate receptors contribute to the development of kindling. We took advantage of the ability of the decahydroisoquinoline LY293558 to selectively block GluR5-containing kainate receptors as well as AMPA receptors. Electrophysiological recordings have demonstrated that LY293558 competitively antagonizes kainate-evoked currents in HEK 293 cells stably expressing homomeric GluR5 kainate receptors (IC$_{50}$, 2.5 μM) but does not affect GluR6 kainate receptor mediated responses (Bleakman et al., 1996, 1999). The selectivity for GluR5 kainate receptors was confirmed in radioligand binding studies with recombinant AMPA and kainate receptors. LY293558 exhibited the highest affinity for GluR5 kainate receptors ($K_{i}$, 4.80 μM) or GluR2 AMPA receptor ($K_{i}$, 3.25 μM) subunits; the drug had lower affinity for GluR1 ($K_{i}$, 9.21 μM), GluR3 ($K_{i}$, 32 μM) and GluR4 ($K_{i}$, 50.52 μM) AMPA receptor sub-
units and was inactive at GluR6 and KA-2 kainate receptors (Simmons et al., 1998). Thus, LY293558 antagonizes AMPA receptors and kainate receptors containing the GluR5 subunit. On an mg/kg basis, GYKI 52466 and LY293558 generally have similar potencies and this was the case with respect to their ability to protect against fully kindled seizures (Fig. 6). However, LY293558 was modestly less effective than GYKI 52466 at retarding the rate at which behavioral kindling developed (Fig. 4 and Table 1). Thus, it seems unlikely that GluR5 kainate receptors are critical to the kindling process.

In conclusion, our results provide support for the concept proposed by Dürrmüller et al. (1994) that AMPA receptors are involved in the expression but not the development of amygdala kindling. GluR5 kainate receptors also do not seem to be required for kindling epileptogenesis in this model. However, in view of the emerging evidence that kainate receptors mediate various forms of synaptic plasticity in brain regions that are relevant to epilepsy (Bortolotto et al., 1999; Li and Rogawski, 1999), the possible involvement of these receptors in the development of other types of epileptic phenomenon is worthy of investigation.

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