Synchronized Oscillations in Hippocampal CA3 Neurons Induced by Metabotropic Glutamate Receptor Activation

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The metabotropic glutamate receptor agonist (1S,3R)-1-aminocyclopentane-1,3 dicarboxylic acid (ACPD) at concentrations above 60 μ M produced stereotypic oscillatory activity in CA3 pyramidal cells of rat hippocampal slices. This oscillatory activity consisted of trains of depolarizations with overriding action potentials. On average, individual trains lasted 7 sec and recurred at intervals of 24 sec. During each train, the constituent depolarizations achieved a maximum frequency of 27 Hz, then slowed to 8 Hz toward the end of the train. Extracellular and dual intracellular recordings suggested that this ACPD-induced oscillatory activity occurred synchronously in the CA3 population. The oscillations persisted in the presence of GABA_A, GABA_B, and NMDA receptor antagonists. In contrast, the oscillations were blocked by the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10–30 μ M). Likewise, the oscillations were blocked by the metabotropic glutamate receptor antagonists (+)- α -methyl-4-carboxyphenylglycine [(+)-MCPG; 1 mM], (S)-4-carboxy-3-hydroxyphenylglycine [(S)-4CPHG; 1 mM] and (S)-4-carboxyphenylglycine [(S)-4CPG; 1 mM]. The results suggest that activation of metabotropic glutamate receptors can result in a permissive state that allows AMPA/kainate receptor-mediated conductances to mediate synchronized activity among hippocampal CA3 neurons.

[Key words: glutamate, metabotropic glutamate receptor, synapse, ACPD, archaocortex, hippocampus, synchrony, oscillations, excitation, inhibition, rhythm, intracellular recordings]

Metabotropic glutamate receptor (mGluR) activation is involved in the generation of distinct patterns of synchronized activity in the hippocampus. Direct activation of mGluRs with ACPD is reported to induce seizures *in vivo* (Sacaan and Schoepp, 1992), and can modulate the *in vitro* epileptiform activities elicited by application of GABA_A receptor antagonists (Burke and Hablitz, 1995; Merlin et al., 1995) or by tetanic stimulation of afferent fibers (Liu et al., 1993). Activation of mGluRs has recently been demonstrated to result in the expression of 40 Hz synchronized

oscillations in inhibitory neurons in the presence of ionotropic glutamate receptor antagonists (Whittington et al., 1995). In this article, we introduce a new phenomenon that is elicited by intense activation of mGluRs. The phenomenon consists of a stereotypic pattern of synchronized oscillation which occurs spontaneously in hippocampal CA3 neurons in the presence of ACPD.

The mGluRs include at least seven receptor subtypes (Abe et al., 1992; Shigemoto et al., 1992; Akazawa et al., 1994; Ohishi et al., 1994). The first group to be recognized includes mGluRs 1 and 5 which are coupled to phosphoinositide (PI) hydrolysis (Houamed et al., 1991; Masu et al., 1991; Abe et al., 1992; Baude et al., 1993). With the exception of mGluR 6, the other mGluRs (subtypes 2 and 3; 4, 7, and possibly 8) are negatively coupled to cyclic AMP formation (Nawy and Jahr, 1991; Tanabe et al., 1992, 1993; Winder and Conn, 1992; Cartmell et al., 1993a, 1994; Duvoisin et al., 1995). Most mGluRs respond to ACPD (Schoepp and Conn, 1993; Chung et al., 1994); however, mGluRs 2 and 3 can be selectively activated by L-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV; Ohfuné et al., 1993; Poncer et al., 1995), while mGluRs 4, 6, and 7 are selectively activated by L-(+)-2-amino-4-phosphonobutyric acid (L-AP4; Akazawa et al., 1994).

Previous studies of mGluRs have emphasized their roles in phenomena such as synaptic plasticity and excitotoxicity (Ito and Sugiyama, 1991; McDonald and Schoepp, 1993; Schoepp and Conn, 1993; Bolshakov and Siegelbaum, 1994; Bortolotto et al., 1994). In addition, activation of mGluRs might be expected to promote synchronized discharges by facilitating excitability and diminishing inhibition (Desai and Conn, 1991; Glaum and Miller, 1993). The excitatory effects of mGluR activation include membrane depolarization secondary to suppression of potassium conductances (Chapack et al., 1990; Stratton et al., 1990; Chapack and Gähwiler, 1991; Miles and Poncer, 1993), induction of burst firing (Zheng and Gallagher, 1991), and potentiation of an afterdepolarization (Constanti and Libri, 1992; Zheng and Gallagher, 1992; Greene et al., 1994). Activation of presynaptic mGluRs can suppress inhibitory synaptic transmission (Hayashi et al., 1994a) by decreasing GABA release (Poncer et al., 1995). However, activation of presynaptic mGluRs can also suppress calcium currents and excitatory synaptic transmission (Forsythe and Clements, 1990; Lester and Jahr, 1990; Baskys and Malenka, 1991; Trombley and Westbrook, 1992; Schoepp and Conn, 1993; Sladeczek et al., 1993).

In this article, we begin to address the generation mechanisms of ACPD-induced oscillations by identifying the synaptic components necessary for its expression. We have also attempted to

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evaluate the involvement of mGluR subtypes by utilizing selective phenylglycine derivatives with agonist and antagonist activities at specific mGluR subclasses (Hayashi, 1994b; Watkins and Collingridge, 1994).

A preliminary version of these findings has been reported in abstract form (Taylor and Wong, 1994).

Materials and Methods

Slice preparation and chamber conditions. Sprague–Dawley rats (15–80 d old) of both sexes were anesthetized with halothane, then surgically decapitated. Brains were placed in ice cold medium containing 0.5 mM Ca^{2+} and 8 mM Mg^{2+} (low Ca^{2+} /high Mg^{2+} solution) for 1 min, then 400 μm transverse sections were sliced from the right hippocampus using a Vibratome (Technical Products International). Slices were then placed on nylon netting in a recording chamber (QL-2, Fine Science Tools) where they were perfused from below with control medium containing (in mM) Na^+ 149, Cl^- 136, K^+ 5, Mg^{2+} 1.6, Ca^{2+} 2, HCO_3^- 26, H_2PO_4^- 1.25, and glucose 11. Perfusion media were bubbled with 95% O_2 /5% CO_2 to maintain the pH near 7.4. The same gas mixture, warmed and humidified, provided the environment for the upper surfaces of the slices. Slices were continuously perfused at 0.2–0.4 ml/min while the chamber was maintained at 34–36°C (TCU-2, Fine Science Tools). Recordings began after 1–2 hr of equilibration for experiments of 2–12 hr duration. Stratum pyramidale was visualized through an upright binocular microscope (SMZ-2B, Nikon) with illumination (Cole Parmer Instruments) from above. ACPD-induced oscillations seemed to be most reliably elicited from slices obtained from the middle one-third of the hippocampus from animals 24–40 d old. Animal care was in accordance with responsible and humane treatment guidelines approved by the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the New York State Department of Health.

Materials. Inorganic salts, glucose, picrotoxin, bicuculline, tetrodotoxin, and halothane were purchased from Sigma. ACPD, L-CCG-, CNQX, 3-(RS-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 2-hydroxysaclofen, (+)-MCPG, (S)-4C3HPG, and (S)-4CPG were purchased from Tocris Neuramin (Bristol, UK) or Research Biochemicals Inc. A generous gift of CGP 35348 was provided by Ciba-Geigy.

Electrophysiological recordings. Electrodes for intracellular and extracellular recording were pulled from glass (1.0 mm O.D., with filament, World Precision Instruments) on a micropipette puller (Sutter Instruments). Intracellular recording electrodes were filled with 2 M potassium acetate; extracellular electrodes were filled with 2 M NaCl. Intracellular electrodes were beveled (Sutter Instruments) to a final tip resistance of 20–110 M Ω . Extracellular electrodes were less than 5 M Ω . Signals from current-clamp recordings were amplified (model M-707; World Precision Instruments; bandpass, 1–30 kHz or wide band) and displayed simultaneously on an IBM-compatible computer running pCLAMP software (Axon Instruments), an oscilloscope (model 2160, BK Precision), and a chart recorder (model TA240, Gould). Data were acquired at a gain of 20 at 0.2–240 msec per point, and stored on disk for off-line analysis. Input resistance was monitored by delivering 0.5 Hz, 0.6 nA, 50–500 msec D.C. current injections (PG-4000 digital stimulator, Neurodata Instruments) through the intracellular recording electrode. Electrodes were placed within about 1.5 mm of each other for dual recordings.

Characterization of train duration, intertrain interval, and depolarization frequency. Data analysis for normative values was limited to 10 experiments in which animals were 22–31 d old, ACPD concentrations of 100–200 μM were used, and continuous digital records (1.6 msec per point; Fetchex, pCLAMP software) were available for six consecutive trains after at least 20, but less than 50, full cycles of recurring trains, and before any other pharmacological manipulation. In the case of drop application, data analysis was limited to seven experiments using 5 mM drops of ACPD, and where digital records (1.6 msec per point; Fetchex, pCLAMP software) were available after at least two repeated applications, but before any other pharmacological manipulation. Data analysis across slices was derived from the medians of individual slices and reported as mean $\pm$ interslice standard deviation. Intertrain intervals were measured from the start of one train to the start of the next. To analyze the effect of GABA $_A$ antagonists, *t* tests were used to compare 46 trains from six slices. For each slice, we determined the median frequency and intertrain quiescent period (from the end of one train to

the start of the next) for four to six consecutive trains before and after antagonist application. Mean values from each slice were used for interslice comparisons using paired *t* tests, with final values reported in the text as the mean of means $\pm$ SEM.

Drug delivery. Pharmacological agents from concentrated stock solutions were applied to the perfusion media with dilutions of 10–2000 or more. Media of altered ionic compositions were applied isosmotically to the perfusion system with observable effects occurring in 3–9 min. Methods of drug delivery included continuous perfusion, transient bath application, or focal drop application. Continuous perfusion with 200 μM ACPD usually produced the oscillations. Depending on flow rate and length and diameter of perfusion tubing, the latency to an observable response to ACPD ranged from 3 to 9 min for continuous perfusion. Transient bath applications were carried out by Eppendorf microapplication directly to the bath (12 μl drops from 25 mm stock, with a well volume of 1.55 ml, yielded an estimated “initial” concentration of 200 μM). The latency to characteristic ACPD effects via transient bath application ranged from 15–40 sec. Focal drop application of ACPD (200–350 μl , 5 mm) most reliably provoked the ACPD-dependent oscillations. Drops were focally applied with a plastic syringe (previously heated and pulled to a thin tip) so that the applied media covered 1–3 mm of CA3. The latency to characteristic ACPD effects in response to drop application ranged from 1–5 sec. Drop application usually required repetitive application as is required in other systems (Cherubini et al., 1991; Sciancalepore et al., 1993). In experiments aimed at examining synaptic transmission and the role of amino acid receptor subtypes, three approaches were used: (1) test media (low Ca^{2+} /high Mg^{2+} , tetrodotoxin, or amino acid receptor antagonists) were continuously perfused for 20–120 min, and then ACPD was introduced by drop application or by addition to the test media. (2) Transient ACPD-induced oscillations were first evoked by drop application during continuous perfusion with control medium, and then following the introduction of and continuous perfusion with test media, ACPD was reapplied by drop application. (3) Ongoing oscillations were maintained by continuous perfusion with ACPD (100–200 μM) in control medium, and then the test media (with ACPD, 100–200 μM) were introduced.

Results

Data are derived from intracellular recordings obtained from 297 neurons from 189 slices. Included among the intracellular records are 63 dual recordings from CA3 neurons from 38 slices, 12 recordings from CA1 neurons in whole slices, 5 from CA1 “mini slices” (CA1 slices with CA2, CA3, and dentate removed), and 4 from CA3 “mini slices” (CA3 slices with CA2, CA1, and dentate removed). Nineteen extracellular recordings were obtained from the CA3 and CA1 regions within stratum pyramidale.

Effects of ACPD on CA3 neurons

Bath perfusion of ACPD (100–200 μM) induced spontaneous oscillatory activity in hippocampal neurons (Figs. 1, 2). This activity consisted of trains of depolarizations with overriding action potentials. The trains recurred at regular intervals (Fig. 1A). In a given experiment, the durations of the first few trains elicited by ACPD and the intervals between them were highly variable. With continued perfusion, the variability decreased and the oscillations settled into a more stereotypic pattern (Fig. 2) which could be maintained for up to 8 hr. In 10 slices, the train duration was 7.4 ± 4.1 sec (mean $\pm$ SD). The intertrain interval was 24.8 ± 10.1 sec (≈ 0.04 Hz). The depolarizations (with overriding action potentials) within each train reached frequencies of 27.4 ± 6.0 Hz early in the train, then slowed to 7.8 ± 3.5 Hz, and had a median frequency of 15.3 ± 1.0 Hz.

Similar activity was transiently evoked by focal drop application of ACPD (200–350 μl , 5 mm). In seven slices, focal application of ACPD elicited 17.1 ± 4.8 cycles (start of one train to the start of the next) of activity lasting 5.4 ± 1.2 min. This activity consisted of trains lasting 4.8 ± 1.2 sec. The trains

Figure 1. ACPD-induced oscillatory activity recorded intracellularly from CA3 cells. *A*, Perfusion with ACPD (200 μ M) produced a stereotypic pattern of oscillation. The oscillations consisted of rhythmically recurring trains of activity. *B*, Two consecutive trains at a faster chart recording speed. *C*, Trains consisted of repetitive depolarizations with overriding action potentials.

recurred at intervals of 16.0 ± 4.9 sec. The depolarizations within these trains began at frequencies as high as 38 Hz and slowed to frequencies as low as 3 Hz.

Effects of hyperpolarization on ACPD-induced oscillatory activity

Injection of hyperpolarizing current suppressed the action potentials within a train and uncovered the underlying depolarizations (Fig. 3*A,B*). Further hyperpolarization progressively increased the amplitude of the depolarizations (Fig. 3*C*). Neither hyperpolarization nor the absence of action potentials had an obvious influence on the intertrain or interdepolarization interval.

ACPD-induced oscillatory activity is synchronized

When ACPD-induced oscillations were elicited in a slice, the activity was observed in all cells impaled in the CA3 region. Figure 4 shows that significant extracellular voltage deflections could be recorded during the oscillatory events, suggesting that the activity occurred simultaneously in a large number of local neurons. Dual CA3 pyramidal cell recordings showed ACPD-induced oscillations were synchronized in each instance ($n = 63$; Fig. 5). Included with these experiments are dual recordings in which one of the pair of intracellular recording electrodes was maintained in a given cell while the other electrode was moved to two to five different sites to record from different cells within the CA3 region ($n = 28$ cells, 7 slices). Synchronous oscillatory

activity was observed between the simultaneously recorded cells with each successive impalement.

ACPD-induced oscillations depend on synaptic activity

To assess mechanisms involved in the generation of ACPD-induced oscillations, slices were perfused with low Ca^{2+} /high Mg^{2+} medium which is expected to block chemical synaptic transmission (Schwartzkroin and Prince, 1978). With control perfusion medium, focal application of ACPD produced oscillatory activity in response to each of nine applications ($n = 4$ slices; Fig. 6*A*). After switching to low Ca^{2+} /high Mg^{2+} medium, ACPD failed to produce the oscillations in response to each of eight drop applications to the above slices (Fig. 6*B*). Upon returning to control perfusion, a single drop of ACPD evoked stereotypical oscillatory activity in each instance (Fig. 6*C*). Additionally, during tonic perfusion with ACPD, ongoing oscillations were reversibly blocked by low Ca^{2+} /high Mg^{2+} medium ($n = 5$ slices).

Further evidence that neuron-to-neuron signaling plays a role in generating these ACPD-induced oscillations was provided by experiments with the sodium channel blocker tetrodotoxin (TTX). Prior to adding TTX (1–2 μ M) to the perfusion medium, focal application of ACPD produced a transient period of oscillatory activity in response to each of 11 applications ($n = 6$ slices; Fig. 7*A*). Once TTX had blocked spontaneously occurring action potentials, ACPD application did not initiate oscillatory activity in response to any of eleven applications (Fig. 7*B*). Ad-

A **B**

Figure 3. Effects of hyperpolarization on ACPD-induced oscillatory activity. *A1*, Stereotypical ACPD-induced oscillations. Downward deflections were the result of current injections (0.5 nA, 80 msec, 0.5 Hz). *A2*, Transient hyperpolarization (indicated by heavy bar) blocked a single action potential without influencing the rhythmicity of the ongoing depolarizations. *B*, Sustained hyperpolarization (−1.3 nA) blocked action potentials without affecting rhythmicity. *C*, Incremental hyperpolarization (from *C1* through *C4*) progressively suppressed the occurrence of action potentials and augmented the amplitude of the underlying depolarizations. The arrows indicate the peak amplitude of the depolarizations.

ditionally, oscillations produced by tonic perfusion with ACPD ceased when 1 μ M TTX was added to the perfusion medium ($n = 3$ slices).

Effects of excitatory amino acid receptor antagonists on ACPD-induced oscillations

The NMDA receptor antagonist CPP (10–30 μ M) did not block ACPD-induced oscillations in any of nine experiments ($n = 5$ slices; Fig. 8*A*). This was true both when the antagonist was applied prior to exposure to ACPD, and when it was applied between and concomitantly with successive applications of ACPD. In contrast, the AMPA/kainate receptor antagonist CNQX (20–30 μ M) prevented the initiation of oscillatory activity by drop application of ACPD in nine experiments ($n = 5$

slices; Fig. 8*B*). Additionally, when oscillatory activity was sustained by continuous bath perfusion of ACPD (100–200 μ M), addition of CNQX alone (10–20 μ M; $n = 3$ slices) to the perfusion media reversibly abolished the ACPD-induced oscillations.

Effects of metabotropic glutamate receptor antagonists on ACPD-induced oscillations

We used several of the recently developed phenylglycine derivatives to study the role of metabotropic glutamate receptors (mGluRs) in the production of the ACPD-induced oscillations. Bath application of (+)-MCPG (1 mM; $n = 5$), a broad spectrum mGluR antagonist (Jane et al., 1993; Schoepp and Conn, 1993; Hayashi et al., 1994), reversibly blocked ongoing oscillations

Figure 2. Characterization of train duration, intertrain interval, and instantaneous frequency of depolarization. Four experiments are shown, with each of four symbols in *A1* corresponding to the same experiment in *A2*. *A*, In response to ACPD (200 μ M), the first several trains showed variable durations (*A1*) and intertrain intervals (*A2*). Train durations and intertrain intervals became more uniform with repeated cycling. *B*, Instantaneous depolarization frequency (calculated from within-train interdepolarization intervals) was variable during the first few trains (*B1*). After repeated cycles of activity, the pattern of instantaneous depolarization became stereotypic. *B2*, Instantaneous frequency (same cell as *B1*) reached a peak early within a given train, progressively slowed, then dropped to zero during the trough of the intertrain interval.

Intracellular and simultaneous field recording

Figure 4. Paired field potential with pyramidal cell recordings show ACPD-induced activity is of a synchronous nature. *A1*, Rhythmic field potentials from hippocampal CA3 region were synchronous with intracellularly recorded activity (2). *B*, Expanded portion of the same recordings as shown in *A*.

produced by continuous perfusion of ACPD. (*S*)-4C3HPG (1 mM; $n = 4$) and (*S*)-4CPG (500 μ M to 1 mM; $n = 5$; Fig. 9) similarly blocked ACPD-induced oscillations. The latter two phenylglycine derivatives are antagonists at mGluRs 1 and 5, but have agonist activity at mGluRs 2 and 3 (Hayashi et al., 1994b). Another mGluR agonist, L-CCG-I, was ineffective at low concentrations (100–300 μ M), but initiated stereotypical oscillations at high concentrations (500 μ M to 2 mM, $n = 4$, data not shown). Oscillations initiated by L-CCG-I were maintained even while titrating its concentration down to very low concentrations (≈ 6 μ M). L-CCG-I has been reported to be more potent than ACPD at mGluRs 1 and 5, yet might preferentially activate mGluRs 2 and 3 at low doses (Watkins and Collingridge, 1994; Pin and Bockaert, 1995).

Effects of GABA receptor antagonists on ACPD-induced oscillations

Experiments were undertaken to determine if inhibition plays a significant role in producing or shaping ACPD-induced oscillatory activity, as it does with similarly appearing oscillations in the thalamus (von Krosigk et al., 1993). Sustained oscillations were first produced by bath perfusion of ACPD. Slices were then exposed to bicuculline (50 μ M; $n = 5$) or picrotoxin (50 μ M; $n = 3$), antagonists of GABA_A receptor-mediated inhibition. Alternatively, to ensure effective suppression of GABA_A receptor-mediated activity (evidenced by the appearance of epileptiform bursts), picrotoxin ($n = 6$) or bicuculline ($n = 2$) was perfused before exposure to ACPD. In neither set of experiments did GABA_A antagonists block the oscillations (Fig. 10A). However, when ongoing oscillations were sustained by the continuous presence of ACPD, the addition of these antagonists to the perfusion medium increased the amplitude of the depolarizations

Paired intracellular recordings

Figure 5. Dual intracellular pyramidal cell recordings from CA3 cells show ACPD-induced activity is highly synchronized. *A*, Cell 1 expressed ACPD-induced activity without action potentials. The trains of activity occurred synchronously with activity recorded from cell 2. *B*, A portion of activity from the same cells as in *A* at higher temporal resolution, revealing that the individual depolarizations within each train were highly synchronized.

Figure 6. ACPD-induced activity is blocked by perfusion with low Ca²⁺/high Mg²⁺ solution. *A*, Prior to manipulation of the perfusion media (control), drop application of ACPD (5 mM, indicated by solid circles) produced oscillatory activity. *B*, Drop application of ACPD could not evoke oscillations in the same cell as in *A* when the perfusion media contained low Ca²⁺/high Mg²⁺. *C*, After reperfusion with control media, drop application of ACPD once again evoked oscillatory activity.

Figure 7. ACPD-induced activity is blocked by TTX. *A*, Drop application of ACPD (5 mM, indicated by *solid circles*) produced stereotypic activity prior to manipulation of the perfusion media (control). *B*, Once TTX had blocked spontaneous activity, ACPD application did not evoke the oscillations. *b*, Current injection (80 msec duration) produced a characteristic charging curve (hyperpolarizing) or calcium spikes (depolarizing), suggesting cell impalement was maintained.

within the trains, decreased the median frequency of the depolarizations within the trains from 15.3 ± 1.0 to 11.7 ± 0.8 Hz (mean $\pm$ SEM; $n = 46$ trains; six slices; $P < 0.03$), and decreased the quiescent period between trains from 20.42 ± 2.83 to 13.57 ± 1.69 sec (mean $\pm$ SEM; $n = 46$ quiescent periods; six slices; $P < 0.04$).

Similar experiments were carried out to assess the role of

GABA_B receptor-mediated inhibition in the generation of ACPD-induced oscillations. After stable oscillations were elicited by ACPD in the presence of GABA_A receptor antagonists, addition of the GABA_B receptor antagonist CGP 35348 (500 μ M to 1 mM) did not have an obvious effect on the oscillations ($n = 8$; Fig. 10*B*). In the absence of GABA_A receptor antagonists, application of CGP 35348 either alone ($n = 3$) or in combination with 2-hydroxysaclofen (another GABA_B receptor antagonist; 1 mM; $n = 2$) also was without an obvious effect on ongoing ACPD-induced oscillations.

Effects of ACPD in the presence of GABA_A antagonists

Before ACPD was introduced, picrotoxin or bicuculline (50 μ M each) elicited spontaneous epileptiform bursts of 120–800 msec duration that recurred regularly every 6–16 sec ($n = 8$ slices; Fig. 11*A*). The interval between these spontaneous bursts gradually decreased to less than 500 msec as the concentration of ACPD was gradually increased (Fig. 11*B*). The duration of these bursts became abbreviated to 50–100 msec when the concentration of ACPD was greater than 50 μ M ($n = 8$ slices). These effects of ACPD on epileptiform bursts produced by GABA_A receptor antagonists are consistent with the findings of Burke and Hablitz (1995) as well as with recent studies performed in this laboratory which focused on the role of mGluR subtypes 2 and 3 in accelerating the rate of these brief duration epileptiform bursts at low concentrations of mGluR agonists (Merlin et al., 1995). Other investigators have reported that acceleration or suppression of epileptiform bursting can be achieved by varying the concentration of ACPD (Sheardown, 1992; Taschenberger et al., 1992; Burke and Hablitz, 1995).

Effects of tetanic stimulation in the presence of GABA_A antagonists and ACPD

Experiments were carried out in the presence of GABA_A antagonists to test the effect of tetanic stimulation while elevating the concentration of ACPD. Tetanic stimulation (4–8 pulses, 50

Figure 8. Effects of ionotropic glutamate receptor antagonists on ACPD-induced oscillatory activity. *A*, After ACPD-induced activity was reliably evoked by drop application (control), the NMDA receptor antagonist CPP was then bath applied for at least 20 min prior to and concomitantly with additional drop applications of ACPD. Inhibition of NMDA receptors with CPP did not block the oscillations. *B*, Blockade of AMPA/kainate receptors with CNQX blocked ACPD-induced oscillatory activity.

Figure 9. *(S)*-4CPG blocks ACPD-induced oscillatory activity. *A*, Continuous perfusion with 200 μ M ACPD produced stereotypical oscillatory activity (control). *B*, ACPD-induced activity was blocked after a few minutes exposure to *(S)*-4CPG, an antagonist at mGluRs 1 and 5. *C*, Oscillatory activity resumed after *(S)*-4CPG was washed out from the perfusion medium.

μ sec pulses, 20–60 Hz) of afferent fibers in the presence of GABA_A antagonists alone evoked epileptiform bursts that were indistinguishable from those occurring spontaneously (Fig. 11*A*). At less than 20 μ M ACPD, tetanic stimulation of afferent pathways failed to produce trains of oscillatory activity in each of 44 trials ($n = 4$ slices). At 20–35 μ M ACPD, tetanization evoked more prolonged discharges that resembled trains in response to 12 of 35 tetanizations (Fig. 11*B*; $n = 4$ slices). At 40–60 μ M ACPD, tetanization produced train-like discharges in response to 19 of 21 tetanizations ($n = 4$ slices). The minimum (“threshold”) concentration of ACPD required to elicit spontaneous trains of oscillatory activity, with or without GABA_A antagonists, was 20–70 μ M ($n = 12$ slices). Tetanization just prior to an expected train, at higher concentrations of ACPD, produced evoked trains that were indistinguishable from spontaneous trains (Fig. 11*C*).

In other experiments, the concentration of ACPD was initially increased to 50 μ M ($n = 3$ slices). This concentration was usually just below threshold for the generation of spontaneous ACPD-induced oscillatory activity. Tetanization of slices under these conditions produced an initial train which could occur in isolation (Fig. 12) or could be followed by up to six spontaneous trains of stereotypical ACPD-induced oscillatory activity (not

shown). Infrequently occurring trains (spontaneous or evoked) tended to be very prolonged (compare Figs. 11, 12).

Since tetanization produced a transient period (one to six trains) of ACPD-like oscillations in the presence of subthreshold concentrations of ACPD, the stimulation likely induced the oscillations by increasing the amount of mGluR activation by the glutamate that was released. Trains of ACPD-like oscillations did not occur in response to tetanization in the presence of MCPG or *(S)*-4CPG.

Discussion

General findings

This article describes a novel form of oscillatory activity that is initiated when the level of mGluR activation is relatively high (generally >60 μ M ACPD). This ACPD-induced oscillatory activity consists of rhythmic (≈ 0.04 Hz) trains of depolarizations (3–38 Hz) which are synchronously generated in neighboring neurons (Figs. 4, 5). The oscillations primarily result from excitatory interactions (Figs. 8, 9), presumably involving recurrent excitatory synapses. GABA receptor-mediated inhibitory interactions play a modulatory role in determining the frequency of the oscillations.

Several lines of evidence suggest that the synchronized de-

Figure 10. Blockade of GABAergic inhibition does not block ACPD-induced oscillatory activity. *A1a*, Oscillatory activity was produced by tonic perfusion with ACPD (200 μM). *A1b*, A single train of activity from the same cell as in *A1a*. *A1c*, Asterisk indicates a portion of activity from the first train in *A1a*. *A2a*, Addition of bicuculline to the perfusion media augmented the amplitude of the oscillatory activity. *B*, In experiments where ACPD and bicuculline and were perfused together (*B1a*, *B1b*), the addition of the GABA_B antagonist CGP35348 (1 mM, *B2a*, *B2b*) for at least an hour did not block the oscillations.

Figure 11. This figure shows paired intracellular recordings from CA3 neurons to compare evoked (tetani indicated by solid circles) and spontaneous activity in the presence of picrotoxin (PTX, 50 μ M) while the concentration of ACPD was increased. **A**, In the presence of PTX alone, evoked bursts resembled spontaneous bursts. **B**, In the presence of 30 μ M ACPD, spontaneous bursts occurred at an accelerated rate and were abbreviated in duration (see text). Accelerated bursting can be seen to the left of the tetanus and in the right half of **B**. Tetanic stimulation evoked a more prolonged synchronized discharge at this concentration of ACPD. **C**, At high concentrations of ACPD (100 μ M), evoked trains resembled spontaneous trains.

PTX + subthreshold [ACPD]

Figure 12. Extracellular stimulation evoked trains similar to ACPD-induced activity when the ACPD concentration was subthreshold for the generation of spontaneous trains. This figure shows paired intracellular recordings from CA3 neurons from a slice initially treated with picrotoxin (PTX, 50 μ M). Three accelerated epileptiform bursts can be seen to the left of the tetanus (tetanus indicated by solid circle). ACPD (50 μ M) was not sufficient to induce spontaneous trains of oscillatory activity in this preparation, yet tetanic stimulation at this concentration evoked a train. Single trains had the characteristics of prolonged ictal-like discharges.

polarizations within each train represent synaptically generated potentials. Firstly, the depolarizations within each train increase in amplitude in response to hyperpolarizing current (Fig. 3), a finding characteristic of chemical synaptic transmission (Hubbard et al., 1969). Secondly, the depolarizations are absent when slices are perfused with a low Ca^{2+} /high Mg^{2+} medium (Fig. 6), or when sodium-dependent action potentials are blocked by TTX (Fig. 7). This suggests that the oscillations are not simply an intrinsic membrane oscillation. Thirdly, the activity is blocked by the AMPA/kainate receptor antagonist CNQX (Fig. 8), suggesting that AMPA/kainate receptors mediate an essential synaptic component required to couple the CA3 neurons.

Pharmacological properties of the ACPD-induced oscillations

Although AMPA/kainate receptor-mediated synaptic transmission is required for generating the synchronized depolarizations that constituted ACPD-induced oscillations, this activity did not occur unless metabotropic agonists were present. The activation of mGluRs appears to be essential to condition the network to generate ACPD-induced oscillations. Since ACPD is an agonist at most mGluR subtypes (Tanabe et al., 1992; Schoepp and Conn, 1993; Chung et al., 1994), the oscillations produced by ACPD could be mediated by PI-coupled mGluRs (mGluRs 1 and 5), and/or by mGluRs negatively coupled to cyclic AMP (mGluRs 2 and 3). There is no evidence as of yet regarding the exact role of the above second messenger systems in generating the ACPD-induced oscillatory activity reported in this article.

The effectiveness of the broad spectrum mGluR antagonist (+)-MCPG in blocking ACPD-induced oscillatory activity strongly suggests a role for mGluRs in the production of the oscillations (Chinestra et al., 1993; Jane et al., 1993; Hayashi et al., 1994b). The antagonist actions of (*S*)-4C3HPG and (*S*)-4CPG on the oscillations suggest that PI-coupled mGluRs are required to generate the oscillations because both of these agents are antagonists at mGluRs 1 and 5, but agonists at mGluRs 2 and 3 (Birise et al., 1993; Eaton et al., 1993; Jones et al., 1993; Hayashi et al., 1994b). The high concentration of ACPD necessary to generate these oscillations also is consistent with the involvement of mGluRs 1 or 5 because molecular and biochemical experiments with cortical tissue suggest that higher levels of glutamate or ACPD are required for activation of mGluRs 1 and 5 than for activation of mGluRs 2 and 3 (Abe et al., 1992; Schoepp et al., 1992; Tanabe et al., 1992; Cartmell et al., 1993a,b, 1994; Challis et al., 1994). Additionally, the concen-

tration of ACPD required to modulate the rate of interictal bursting through activating mGluRs 2 and/or 3 (Merlin et al., 1995) is significantly lower than that required to initiate the prolonged discharges reported herein. While the above discussion strongly suggests a role for PI-coupled mGluRs, this certainly does not rule out a role for other mGluRs. One role for other subtypes could be that their activation may reduce inhibition to a level that would permit synchronization. The role of mGluRs 2 and 3 in reducing inhibition has been studied in detail by Poncer et al. (1995).

How do ACPD-induced oscillations compare with other similarly appearing oscillations?

The oscillatory activity reported in this article is a new finding and may be related to other similarly appearing oscillations which have been studied in some detail. Two distinct features of the ACPD-induced oscillations are the following. (1) Each train consists of synchronized repetitive depolarizations at frequencies up to 38 Hz. (2) The trains of depolarizations recur every 10–50 sec, separated by quiescent periods. Similarly, thalamocortical spindle oscillations (Steriade et al., 1985, 1990, 1993c; Buzsáki et al., 1988; von Krosigk et al., 1993), hippocampal rhythmic slow activity *in vitro* ("RSA"; Bland, 1986; MacVicar and Tse, 1989), and slow neocortical oscillations (Steriade et al., 1993a) all consist of periodic clusters of depolarizations. Do ACPD-induced oscillations require the same set of synaptic components as any of the above oscillations?

Experiments *in vitro* have demonstrated that thalamic spindle oscillations (von Krosigk et al., 1993) and hippocampal RSA (MacVicar and Tse, 1989; Traub et al., 1992) both are sensitive to AMPA/kainate receptor antagonists, as are the ACPD-induced oscillations reported in this article. Slow neocortical oscillations, on the other hand, are suggested to be modulated by NMDA receptor mediated activity (Steriade et al., 1993a). Thalamic spindle oscillations are distinctive in that they are blocked by the combined application of antagonists for GABA_A and GABA_B receptor-mediated inhibition (von Krosigk et al., 1993). In contrast, antagonists of GABA receptor-mediated inhibition have no effect on *in vitro* RSA (MacVicar and Tse, 1989; Traub et al., 1992) and do not block the ACPD-induced oscillatory activity reported in this article.

The intertrain interval. Steriade et al. (1993a) suggest that synaptic inhibition mediated by a chloride conductance contributes to determining the length of the quiescent period between

trains of the slow neocortical rhythm. In our studies, GABA_A receptor antagonists decreased the duration of the quiescent period between trains from 20 to 14 sec. This finding is consistent with a modulatory role for GABA_A receptors in shaping ACPD-induced oscillations, and distinguishes this activity from carbachol-induced RSA recorded *in vitro* which is unaffected by GABA antagonists (MacVicar and Tse, 1989). A role for fast inhibition is not surprising because GABA_A receptor-mediated inhibition develops rapidly enough to influence the process of synchronization (Traub et al., 1987; Wong and Miles, 1993).

At present, the exact ionic basis for slow rhythms is unknown. With regard to thalamic spindling, the oscillations depend on rebound bursting involving a low threshold calcium conductance (I_T), while train termination involves interactions between I_T and the depolarizing influence of I_h (McCormick and Pape, 1990; Soltesz et al., 1991; Huguenard and McCormick, 1992; McCormick and von Krosigk, 1992). In contrast, ACPD-induced oscillations do not appear to involve rebound bursting, and the termination of each train is accompanied by a hyperpolarization (Alger and Williamson, 1988) followed by a slow depolarization that is reminiscent of a pace-making current (Leresche et al., 1990; DiFrancesco, 1991; Huguenard and McCormick, 1992).

Intratraining depolarizations. Our experiments with ACPD-induced oscillations demonstrate a predominant role for glutamate receptor-mediated conductances (Jonas and Spruston, 1994; Westbrook, 1994) in the CA3 region. ACPD-induced oscillations were present in CA3 "mini slices" but absent in CA1 "mini slices" (data not shown). This is consistent with the known function of CA3 recurrent synapses (Miles and Wong, 1986; Ishizuka et al., 1990) which generate synchronized discharges in the hippocampal slice (Traub and Wong, 1982; Wong and Traub, 1983; Traub and Miles, 1991; Bianchi and Wong, 1994).

GABA_A receptor antagonists had the dual effect of enhancing the amplitude of the depolarizations while decreasing the median frequency of depolarization from 15.3 to 11.7 Hz. This was particularly true when the depolarizations were of low amplitude and the trains recurred infrequently. These findings are consistent with a role for GABA_A receptors in modulating ACPD-induced oscillations. The results also suggest that inhibition was not completely blocked when ACPD-induced oscillatory activity occurred. The modulatory role of residual inhibition in shaping ACPD-induced oscillations resembles the findings of von Krosigk et al. (1993) who report that thalamic spindles are also augmented and slowed by GABA_A antagonists. However, in contrast to the generation of thalamic spindle oscillations which require excitatory-inhibitory interactions (Jahnsen and Llinás, 1984a,b; Steriade et al., 1985; Buzsáki et al., 1988; von Krosigk et al., 1993; Wang and Rinzel, 1993; Bal et al., 1995a,b), generation of ACPD-induced oscillations appear to require excitatory-excitatory interactions and are only modulated by residual tonic or phasic inhibition.

General conclusions

Clusters of synchronized depolarizations are a fundamental manifestation of interactions in neural circuitry and can be expressed during physiological or pathophysiological events. Different circuitries can generate similar patterns of activity yet possess different synaptic and intrinsic mechanisms. The present study suggests that ACPD-induced oscillations, slow neocortical rhythms (Steriade, 1993a-c), spindle waves (von Krosigk et al., 1993), and carbachol-induced RSA (MacVicar and Tse, 1989) result from distinct yet overlapping mechanisms. Our findings pro-

duced *in vitro* in the continuous presence of mGluR activation are probably most relevant to the pathophysiology of epileptiform discharges (Miles et al., 1984; Swann et al., 1986, 1993; Traub et al., 1991). During the course of pathophysiological brain activity, mGluR activation could transiently reach a level such that an ictal discharge similar to the trains of depolarizations reported here might occur. The results we have presented may lead to an understanding of the mechanisms underlying the transition from interictal to ictal discharge.

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