Enhancement of Synaptic Excitation by GABA\textsubscript{A} Receptor Antagonists in Rat Embryonic Midbrain Culture

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Rohrbacher, Jutta, Katja Sauer, Andrea Lewen, and Ulrich Misgeld. Enhancement of synaptic excitation by GABA\textsubscript{A} receptor antagonists in rat embryonic midbrain culture. J. Neurophysiol. 79: 1113–1116, 1998. Alterations of synaptic excitation induced by exposure to \(\gamma\)-aminobutyric acid-A (GABA\textsubscript{A}) receptor antagonists were investigated employing tight-seal whole cell recording from single neurons or pairs of neurons in rat embryonic midbrain culture. Application of GABA\textsubscript{A} receptor antagonists led to sustained depolarizations followed by synchronous paroxysmal depolarization shifts (PDSs). PDSs induced a transient increase in miniature excitatory postsynaptic currents in the presence as well as in the absence of a \(N\)-methyl-\(d\)-aspartate receptor antagonist. The increase in glutamate release supports the excitatory drive required to reinitiate PDSs from the quiescent interburst intervals. After washout of GABA\textsubscript{A} receptor antagonists, synaptic activity remained grouped, regardless of the presence or absence of PDS blockade by tetrodotoxin (TTX). Impediment of action potential-triggered transmitter release by Cd\textsuperscript{2+} or TTX also induced grouped activity. We conclude that changes in synaptic excitation are produced by the impaired GABA\textsubscript{A} inhibition per se and by the initiation of PDSs.

INTRODUCTION

Cellular mechanisms underlying the generation of epileptiform activity have been investigated in various ‘‘in vitro’’ preparations, including primary brain cell cultures (Maeda et al. 1995; Murphy et al. 1992; Segal and Furshpan 1990). Periodic bursts occurring in embryonic neuronal cultures after blockade of \(\gamma\)-aminobutyric acid-A (GABA\textsubscript{A}) inhibition are not controlled by specific pacemaker cells but rather are produced by spatial and temporal summation of synaptic events (Müller and Swandulla 1995). ‘‘Miniature’’ synaptic potentials could contribute to the excitatory drive to reinitiate bursts that have been terminated as a result of, for example, afterhyperpolarization, receptor desensitization, and refractoriness. Moreover, synchronous bursts may induce long-lasting changes that apparently involve plasticity of neuronal connections (Forti et al. 1997; Schneidermann et al. 1994). Activation of glutamate receptors induces long-term potentiation of the frequency of miniature synaptic currents in cultured hippocampal neurons by an alteration of the machinery controlling transmitter release (Malgaroli and Tsien 1992). The present study was undertaken to address the question whether such a process could control the burst activity in a cultured embryonic network of the midbrain.

METHODS

Fourteen-day-old rat embryos were removed from Wistar rats that had been anesthetized by inhalation of ether and killed by decapitation. Pieces of ventral midbrain tissue were dissociated mechanically and plated on a primary culture of glial cells from the same area. Culturing techniques were as previously described (Bijak et al. 1991; Jarolimek and Misgeld 1992; Jarolimek et al. 1996; Rohrbacher et al. 1997). All data reported here were from neurons that have been cultured for \(\approx\)3 wk. Tight-seal whole cell recordings were made at room temperature (20–24°C). We recorded synaptic currents in a discontinuous voltage-clamp mode with a single-electrode voltage-clamp amplifier (SEC-II, npi, Tamm, Germany). Data from experiments were accepted for analysis only as long as access resistance and input resistance remained constant.

The extracellular solution contained (in mM) 156 NaCl, 2 KCl, 2 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, 15 glucose, 10 \(N\)-2-hydroxyethylpiperazine-\(N'\)-2-ethanesulfonic acid (HEPES), and 0.001 glycine at pH 7.3. Recording electrodes (resistance to bath 3–5 M\(\Omega\)) were filled with (in mM) 10 NaCl, 120 KCl, 5 ethylene glycol-bis(\(\beta\)-aminoethyl ether)-\(N,N,N',N'\)-tetraacetic acid, 0.25 CaCl\textsubscript{2}, 10 glucose, 10 HEPES, and 2 Mg-ATP at pH 7.3. For low \([\text{Cl}^-]_{\text{intr}}\), KCl was replaced by 100 mM K-glutconate and glucose increased to 40 mM. The recording chamber was perfused continuously with control or drug containing solution (volume of the chamber: 700 \(\mu\)l, perfusion rate: 0.6 ml/min). To analyze miniature excitatory postsynaptic currents (mEPSCs) data were stored on a DAT-recorder and sampled and digitized with 2 kHz using a CED interface and EPC software (CED, Cambridge, UK) or digidata hardware and pClamp software 6.0 (Axon Instruments, Foster City, CA). Details for the analysis of mEPSCs were described elsewhere (Jarolimek and Misgeld 1997; Rohrbacher et al. 1997). The program used for analysis detects events if the difference between the baseline (data averaged for 2 ms) and a following (1.5–2 ms) current value exceeds a given threshold (8–12 pA) during a time period of 2 ms. Superimposed events with a latency of >2 ms were detected separately and measured. Each event was inspected visually before being accepted. mEPSCs analyzed this way were blocked reversibly by 6,7-dinitroquinoxaline-2,3-dione (DNQX, 1 \(\mu\)M). Differences in amplitude and frequency distribution were tested for statistical significance using the Kolmogorov-Smirnov test (K-S, \(P < 0.01\)).

RESULTS

Superfusion of the GABA\textsubscript{A} receptor antagonists (+)-bicuculline or picrotoxin (20 \(\mu\)M) induced a rather stereotyped sequence of events (Fig. 1A). Cells depolarized abruptly. After a sustained depolarization (SD), recurrent paroxysmal depolarizing shifts (PDSs) developed that occurred synchronously in cell pairs (distance \(\approx\)100 \(\mu\)m, \(n = 15\)) regardless whether the pairs were synaptically coupled (Fig. 1B) or not. Voltage-clamp recording (high \([\text{Cl}^-]_{\text{intr}}\)) revealed the intense inhibitory synaptic barrage (Fig. 2), which bombarded the cells before GABA\textsubscript{A} receptor antagonists were...
Sums of the current amplitudes in 5-s periods (Fig. 3B) revealed an increase after the PDSs in 11 of 15 cells. The activity increase lasted 142 ± 26.5 s (n = 9) after the last burst. In three cells, the test was repeated after 5 min, resulting in a renewal of the increase in synaptic activity in two cells. In another two cells, the enhancement of mEPSCs persisted for 5 min. An additional period of PDSs could not increase mEPSCs further. For statistical analysis, mEPSCs were sampled during 1-min periods before and after PDSs (20–80 s). Applying this analysis, we may have underestimated the change in mEPSCs because mEPSCs could come back to control values during the sampling period. Yet, in 9 of 15 cells there was a significant increase in the frequency of mEPSCs (Fig. 3, D and E, K-S, P < 0.01, mean frequency: 10.7 ± 4.7 Hz before, 22.9 ± 5.3 Hz after PDSs). In four of the nine cells, the increase in the frequency was associated with an increase in amplitudes (K-S, P < 0.01), however, in three of four cells, the frequency was so much enhanced that mEPSCs could not always be separated reliably. Thus the increase in amplitude partly may be due to an increase in frequency. The mean amplitudes of mEPSCs of the cells in which the frequency was increased was not changed (20.1 ± 1.3 pA before and 21.2 ± 2.1 pA after PDSs, n = 9). NMDA receptors were not required to increase the frequency of mEPSCs because 4-Methyl-APPA did not prevent the effect (Fig. 3, A and B, n = 3 of 10 cells). The increase in frequency was associated with an increase in amplitude in two of the three cells (K-S, P < 0.01). The only influence of NMDA receptors was a smaller increase in synaptic activity after PDSs as compared with the time before PDSs (Fig. 3C). Action potential-independent constitutive glutamate release could contribute to synaptic noise. Therefore, we tested whether the increase in synaptic activity after PDSs is associated with an increase in the frequency of mEPSCs.

We recorded mEPSCs in the presence of TTX (0.3 μM) or, in some cases, of TTX and Cd^{2+} (20 μM) and compared the frequencies and amplitudes of mEPSCs before and after the occurrence of PDSs. Toward this end, we first exposed the culture for 5 min to TTX (and Cd^{2+}) and picrotoxin to sample mEPSCs. Thereafter TTX (and Cd^{2+}) was washed out with picrotoxin-containing solution to let the network generate PDSs. Time required for generating PDSs was 2–8 min. PDSs were stopped by the reaplication of TTX (and Cd^{2+}); this allowed us to resample mEPSCs (Fig. 3A).

Data described before indicate that the development of PDSs during pharmacological blockade of GABA_A inhibition is dependent on the strength of synaptic excitation. The quiescent states between PDSs are characterized by synaptic noise. Synaptic noise was more pronounced immediately after PDSs as compared with the time before PDSs (Fig. 3C). Action potential-independent constitutive glutamate release could contribute to synaptic noise. Therefore, we tested whether the increase in synaptic activity after PDSs is associated with an increase in the frequency of mEPSCs.

FIG. 1. Induction of epileptiform activity in pairs of midbrain neurons by γ-aminobutyric acid-A (GABA_A) receptor blockade. A: bicuculline (Bic, 20 μM, arrow bar indicates start of perfusion with Bic) induced a sustained depolarization (SD) and synchronized paroxysmal depolarization shifts (PDSs, low [Cl-]_o). Lines and numbers on top of the continuous chart record indicate time periods at which depolarizing current pulses were injected into the cells to test for synaptic coupling. B: action potentials in each cell triggered excitatory postsynaptic potentials (EPSPs) in the other cell.

applied (n = 33). SDs and PDSs were blocked by tetrodotoxin (TTX, 0.3 μM, Figs. 2B and 3A, n = 29) or the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist DNQX (0.65 ± 1 μM, n = 6). In low concentration (0.3 μM), DNQX prolonged the period until SDs and PDSs appeared (360–540 s, n = 4 in DNQX and picrotoxin; 99.5 ± 10.6 s, mean ± SE, n = 21, in picrotoxin). SDs and PDSs were not blocked by the high affinity N-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-4-methyl-5-phosphono-3-pentenoic acid (4-Methyl-APPA, 1 μM), however, time to onset of SDs was prolonged (205.5 ± 50.6 s, n = 10) and the duration of PDSs shortened (n = 12).

FIG. 2. Enduring changes in the network excitability after blockade of GABA_A receptor activity increase lasted 142 ± 26.5 s (n = 9) after the last burst. In three cells, the test was repeated after 5 min, resulting in a renewal of the increase in synaptic activity in two cells. In another two cells, the enhancement of mEPSCs persisted for 5 min. An additional period of PDSs could not increase mEPSCs further. For statistical analysis, mEPSCs were sampled during 1-min periods before and after PDSs (20–80 s). Applying this analysis, we may have underestimated the change in mEPSCs because mEPSCs could come back to control values during the sampling period. Yet, in 9 of 15 cells there was a significant increase in the frequency of mEPSCs (Fig. 3, D and E, K-S, P < 0.01, mean frequency: 10.7 ± 4.7 Hz before, 22.9 ± 5.3 Hz after PDSs). In four of the nine cells, the increase in the frequency was associated with an increase in amplitudes (K-S, P < 0.01), however, in three of four cells, the frequency was so much enhanced that mEPSCs could not always be separated reliably. Thus the increase in amplitude partly may be due to an increase in frequency. The mean amplitudes of mEPSCs of the cells in which the frequency was increased was not changed (20.1 ± 1.3 pA before and 21.2 ± 2.1 pA after PDSs, n = 9). NMDA receptors were not required to increase the frequency of mEPSCs because 4-Methyl-APPA did not prevent the effect (Fig. 3, A and B, n = 3 of 10 cells). The increase in frequency was associated with an increase in amplitude in two of the three cells (K-S, P < 0.01). The only influence of NMDA receptors was a smaller percentage of cells in which a change after the PDSs was observed.

Transient exposure (3–10 min) to GABA_A receptor an-
agonists induced enduring changes in the excitability of the network. After washout of the GABA_A receptor blockers synaptic activity remained grouped (Fig. 2A). Inhibition was operating because reapplication of picrotoxin produced PDSs again (Fig. 2A). Synaptic activity remained clustered even if the cultures were perfused with picrotoxin and TTX (Fig. 2B, 8–10 min, n = 7). TTX prevented the occurrence of SDs and PDSs. mEPSC frequency did not change during exposure to picrotoxin and TTX. Thus blockade of GABA_A receptors induces changes in the network excitability through effects additional to modifications induced by PDSs as, for example, the increase in the frequency of mEPSCs. Further, changes were not caused solely by the drugs used for GABA_A receptor blockade. Blockade of inhibition itself induced some changes. Reducing action potential-dependent transmitter release and, thereby, inhibition (as well as excitation) produced some grouping of synaptic activity. The change was observed in two of three cells after exposure to Cd^{2+} (10 μM) for slightly longer time periods (Fig. 2C, 10–15 min) and in two of four cells after exposure to TTX (0.3 μM). Cd^{2+} (10 μM) reduced action potential-dependent synaptic currents but did not block GABA_A receptors. Only a high concentration of Cd^{2+} (100 μM) reduced GABA_A receptor-induced currents (10 μM, n = 4; 100 μM, n = 5, data not shown).

**DISCUSSION**

Synchronous PDSs were recorded in cultured cell pairs during GABA_A receptor blockade regardless whether the cells in the pair were synaptically coupled or not. For cell pairs not synaptically coupled, therefore, a third cell or, more likely, the cell aggregate must provide the synchronous excitatory drive. The assumed synchrony in the cell aggregate is in good agreement with the synchrony of intracellular Ca^{2+} oscillations, which were induced by GABA_A receptor blockade in a comparable network of cultured hypothalamic cells (Müller and Swandulla 1995). We could detect increases in mEPSCs that were induced by epileptiform activity after GABA_A receptor blockade in some cells. Changes in mEPSC frequency and changes in action potential-dependent responses occur usually in parallel (Malgaroli and Tsien 1992), suggesting that the change in mEPSCs observed here may contribute to the epileptogenic action of GABA_A receptor blockade. Many studies reported a major role for NMDA receptors in the induction of excitability changes by epileptiform activity (Dingledine et al. 1990; Lee and Hablitz 1991; Traub et al. 1993). In rat embryonic midbrain culture, the effect largely was mediated by AMPA receptors. In the cultured network, NMDA receptors were not required to generate PDSs, however, they contributed to the excitatory drive. If, on the other hand, the perfusate contains high levels of divalent cations, NMDA receptor-mediated effects are not at all observed although the network can generate burst activity (Jarolimek and Misgeld 1992; Müller and Swandulla 1995). Provided conditions were employed in cultured networks that allowed stronger activation of NMDA receptors than occurred under the conditions chosen here, activation of NMDA receptors during PDSs well might induce additional frequency dependent effects. Our data further show that the


The epileptogenic potency of GABA \(_A\) receptor blockers results from the blockade of GABA \(_A\) inhibition per se and from changes in excitatory drive induced by the epileptiform activity.

References


