Taira, Tomi, Karri Lamsa, and Kai Kaila. Posttetanic excitation mediated by GABA A receptors in rat CA1 pyramidal neurons. J. Neurophysiol. 77: 2213 ± 2218, 1997. The contributions of γ-amino- butyric acid (GABA) receptors to posttetanic excitation of CA1 pyramidal neurons in rat hippocampal slices were studied using extracellular and intracellular recording techniques. Synaptic responses were evoked on tetanic stimulation (100–200 Hz, 40–100 pulses) applied in stratum radiatum close (300 ± 600 μm) to the recording site. Under control conditions, tetanic stimulation resulted in a triphasic depolarization/hyperpolarization/sustained depolarization sequence in area CA1 pyramidal cells. The late depolarization usually gave rise to a prolonged (≤3 s) spike firing. The late depolarization and the associated spike firing were blocked both specifically and completely (within a time window of 3–6 min starting from picrotoxin application) by the GABA A receptor antagonist picrotoxin (PiTX, 100 μM). Paradoxically, at this early stage of PiTX application, overall neuronal firing was attenuated to a higher degree than what was achieved by ionotropic glutamate antagonists. Complete block of ionotropic glutamate receptors by the antagonists D-2-amino-5-phosphonopentoate (AP5, 80 μM), 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (NBQX, 10 μM), and ketamine (50 μM) blocked the initial fast depolarization and suppressed the late one. Exposure to a permeable inhibitor of carbonic anhydrase, ethoxyzolamide (EZA, 50 μM) inhibited the late, apparently GABA-mediated inhibitory postsynaptic potentials. The excitatory actions of GABAergic interneurons may accentuate the excitation of principal neurons and, hence, play an important facilitatory role in the induction of long-term potentiation (LTP) and epileptogenesis.
FIG. 1.  A: intracellular recording of a typical triphasic response in CA1 pyramidal cell evoked on afferent stimulation (200 Hz/0.5 s) applied to stratum radiatum under control conditions (left). After a 5-min wash-in of 100 μM picrotoxin (PiTX), late depolarization and associated spike firing are blocked, whereas hyperpolarizing component is yet almost unaffected (middle). Prolonged (10 min) application of PiTX eventually leads to a disappearance of the γ-aminobutyric acid-A (GABA_A) receptor-mediated inhibitory postsynaptic potentials (IPSPs) and thus to an increase in glutamate receptor-mediated excitability (right).  

B: responses to single stimuli before and after PiTX wash-in.  

C: corresponding recordings of extracellular potential transients in stratum radiatum of CA1 region. Timing of stimulus trains is indicated by horizontal bars (A and C) or by arrows (B).

It is also important to note that the stimulation protocols used here are essentially the same as the ones often used for the induction of long-term potentiation (LTP) of
excitatory postsynaptic potentials (EPSPs) (see Bliss and Collingridge 1993) in a number of studies and not the type employed in kindling studies to evoke epileptiform afterdischarges (i.e., a number of successive tetanic stimuli given in a low Mg²⁺ medium and employment of long trains of stimuli with extended pulse duration) (cf. Higashima et al. 1996; Hochman et al. 1995).

Under control conditions, tetanic (100–200 Hz/0.4–0.5 s) stimulation applied to s. radiatum typically resulted in a triphasic depolarization/hyperpolarization/sustained depolarization sequence (n = 12) (Fig. 1A, left). The initial depolarization usually lasted for ≈40 ms, giving rise to only a few spikes. It was followed by hyperpolarization, which then gave way to the late depolarization. The late depolarization had a time to peak of 800–1,200 ms and an amplitude ranging from 5 to 20 mV, giving rise to a prolonged train of spikes, occasionally lasting ≈3 s.

After wash-in of the GABAₐ receptor antagonist, PiTX (100 μM), the late depolarization and spike firing were blocked selectively. Attenuation of the late, apparently GABAₐ receptor-mediated excitation [hereafter termed GABAₐ-mediated depolarizing postsynaptic potential (GDPS)] took place gradually within a time window of 3–6 min from start of PiTX application, during which time the hyperpolarizing component and the fast depolarization remained unchanged (Fig. 1A, middle) (cf. Alger and Nicoll 1982). Thereafter, in parallel with the disappearance of inhibitory postsynaptic potentials (IPSPs) the fast depolarization and associated spike firing started to get accentuated (Fig. 1A, right). Paradoxically, at the early stage of PiTX application, there was a dramatic attenuation of neuronal firing. Figure 1B shows responses to single stimuli in control conditions and in PiTX. Note that the effect of PiTX on single responses is seen only after prolonged application (cf. Fig. 1A). In Fig. 1C, corresponding extracellular responses recorded from stratum radiatum are shown. Extracellular recordings were made in CA1 stratum radiatum due to the dendritic site of origin of the depolarizing GABA responses (e.g., Staley et al. 1995). Similar results were obtained in all six slices exposed to PiTX. On return to control solution, an opposite sequence of events could be seen (not illustrated).

Complete pharmacological block of ionotropic glutamate receptors (10 μM NBQX, 80 μM AP5, 50 μM ketamine) blocked the initial fast depolarization (Fig. 2A). It also diminished the GDPSP, although it was possible to restore the late component by increasing the stimulus intensity/frequency. Postsynaptic responses evoked by single stimuli before and during the drug application are shown in Fig. 2B.

In neurons where E_Cl is more negative than the resting membrane potential, GABAergic depolarizing potentials are dependent on an inwardly directed HCO₃⁻ current (Kaila and Voipio 1987; Kaila et al. 1993). Furthermore, it has been shown that pharmacological inhibition of carbonic anhydrase attenuates the GABAₐ receptor-mediated depolarization in pyramidal neurons (Grover et al. 1993; Staley et al. 1995). In agreement with this, the permeable inhibitor of CA, EZA (50 μM) applied in bath reduced both the amplitude and the length of GDPSP (Fig. 3). However, the effect of EZA was not fully selective with respect to the GDPSP, because the glutamatergic excitation also was diminished (Fig. 3B).

The remaining component of the GDPSP was blocked completely on exposure to PiTX (100 μM).

**Discussion**

It generally is agreed that exposure to GABAₐ receptor antagonists enhances neuronal excitability, often to a degree sufficient to induce epileptic activity (see Krnjevic 1984; Schwartzkroin 1983). Hence, we were rather surprised by the strong attenuation of neuronal firing by PiTX under control conditions. Although temporally limited, the action of PiTX was impressively clear cut. Typically, after a 5-min PiTX wash-in the GDPSP and the associated spike firing were eliminated completely. At this stage, the hyperpolarizing GABAₐ component still was unaffected, and thus the glutamate-mediated depolarization remained curtailed. On continuation of PiTX application, the GABAₐ-mediated IPSPs gradually faded away thus accentuating the glutamatergic excitation.

Why was the GDPSP diminished by PiTX before the hyperpolarizing component was affected? Alger and Nicoll (1982) showed that pentobarbital-induced depolarizing GABA responses were blocked by dendritic application of bicuculline methiodide whereas the hyperpolarizing IPSPs remained intact, thus suggesting different sites of origin (dendritic versus somatic) of the depolarizing and hyperpolarizing components (see also Michelson et al. 1991). It is possible that the selective depression of GDPSP was due to nonuniform distribution of PiTX over the slice. Given the larger fractional volume of the extracellular space in s. radiatum versus s. pyramidale (see Heinemann et al. 1990), one could assume that effective concentration of the drug would be reached more readily in the dendrites. However, it is noteworthy that the interneurons within the hippocampal inhibitory network are excitatory coupled by GABA (Michelson and Wong 1991, 1994). Therefore, we have put forward the following hypothesis (Voipio et al. 1996): An intense afferent stimulation leads to a sustained elevation in [K⁺], thus attenuating the GABAₐ receptor-mediated depolarization in Neurons. By wash-in of the GABAₐ receptor antagonist in kindled hippocampal slices was reported recently by Higashima et al. (1996). However, because of the extracellular recording technique used in the study, they were not able to see the selective effect of the GABAₐ receptor blockade on the depolarizing GABA responses, which is the central finding in our study.

The effect of the glutamate receptor antagonists on GDPSP can be explained by the fact that in the area CA1 there is a strong excitatory drive to the GABAergic interneurons mediated by N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid-type glutamate.
FIG. 2. A: effect of d-2-amino-5-phosphono-pentoate (80 μM), ketamine (50 μM), and 6-nitro-7-sulphamoylbenzo[f]quinoxaline-2,3-dione (10 μM) on postsynaptic responses evoked by tetanic stimulation (100 Hz/0.4 s) applied to s. radiatum. Initial fast depolarization is abolished completely, whereas a substantial part of late component is preserved. Note also that effect on GABA-mediated depolarizing postsynaptic potential (GDPSP) is qualitatively completely different from what is achieved by PiTX (Fig. 1A). B: single stimuli evoked responses (indicated by arrowheads) in absence and presence of ionotropic glutamate blockers.

receptors (cf. Davies et al. 1990). Thus blockade of glutamate-mediated excitation will inevitably lead to a decrease in the di- or polysynaptic GABAergic drive to the pyramidal cells. Reestablishment of the GDPSP by increasing the stimulus frequency or intensity (and thus increasing the direct stimulation of interneurons) after the application of glutamate receptor antagonists supports this explanation.

Depolarizing GABA responses in pyramidal cells are decreased on application of carbonic anhydrase inhibitors, such as acetazolamide (Grover et al. 1993; Staley et al. 1995). Also, in our experiments, blockade of intracellular carbonic anhydrase activity by EZA markedly attenuated GDPSPs. Usually it was not possible to restore the GDPSP by increasing the stimulus intensity. It should be noted, however, that EZA also had an inhibitory effect on the initial glutamatergic excitation. As already pointed out, this can decrease the GABAergic drive to principal cells. The inhibitory effect of EZA on EPSPs could be attributed to acidic shifts in the steady state pH_i and pH_o, which are known to damp overall excitability (see Lee et al. 1996). The posttetanic excitation remaining in the presence of EZA was abolished on PiTX.

Nerve cells can undergo various types of long-lasting changes (e.g., LTP) in their synaptic strength after high-frequency afferent activation. Depolarization or intense bursting of a neuron (regardless of whether it is induced anti- or orthodromically) during simultaneous activation of
Afferent fibers can alter dramatically the way the synaptic connections are affected by the afferent activity (Jester et al. 1995; Kelso et al. 1986). Furthermore, postsynaptic depolarization per se can result in persistent modulation of both EPSPs as well as IPSPs even without temporally coincident afferent activity (Pitler and Alger 1992; Wyllie et al. 1994). Therefore, GDPSPs can serve as a modulatory mechanism for changes in postsynaptic responses after tetanic stimulation. In this regard, it is noteworthy that GDPSPs can induce a neuronal influx of Ca$^{2+}$ (on which most of the plastic changes in postsynaptic neurons are dependent on) in adult rat hippocampal slices (Voipio et al. 1996). Intriguingly, it was shown recently by Frey et al. (1996) that blockade of the GABA$_A$ receptor-mediated transmission may attenuate tetanus-induced LTF in mouse hippocampus. The mechanism underlying this curious finding was not clarified, but a GABAergic mechanism of excitation, such as the one described presently, may well have contributed to it.

The present results may warrant a reinterpretation of the role of GABAergic interneurons in the control of pyramidal cell excitation: during intense interneuronal activity, GDPSPs will be generated, which will assist the ‘conventional’ glutamatergic inputs in the encoding of the strength and duration of afferent excitation. Here we show that GABAergic and glutamatergic mechanisms can act in concert to entrain postsynaptic discharges.

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