Synaptic Activation of GABA<sub>A</sub> Receptors Induces Neuronal Uptake of Ca<sup>2+</sup> in Adult Rat Hippocampal Slices

ANNA-MAIJA AUTERE, KARRI LAMSA, KAI KAILA, AND TOMI Taira

Department of Biosciences, Division of Animal Physiology, University of Helsinki, FIN-00014 Helsinki, Finland

Autere, Anna-Maija, Karrri Lamsa, Kai Kaila, and Tomi Taira. Synaptic activation of GABA<sub>A</sub> receptors induces neuronal uptake of Ca<sup>2+</sup> in adult rat hippocampal slices. J. Neurophysiol. 81: 811–816, 1999. Synaptically evoked transmembrane movements of Ca<sup>2+</sup> in the adult CNS have almost exclusively been attributed to activation of glutamate receptor channels and the consequent triggering of voltage-gated calcium channels (VGCCs). Using microelectrodes for measuring free extracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>e</sub>) and extracellular space (ECS) volume, we show here for the first time that synaptically stimulated by the membrane-permeant inhibitor of carbonic anhydrase, ethoxzolamide (50 μM) or in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)–buffered HCO<sub>3</sub>⁻-free solution. Neuronal Ca<sup>2+</sup> uptake caused by intense synaptic activation of GABA<sub>A</sub> receptors may prove to be an important mechanism in the modulation of activity-dependent neuronal plasticity, epileptogenesis, and cell survival in the adult brain.

INTRODUCTION

γ-Aminobutyric acid (GABA) does not act solely as an inhibitory transmitter in the adult mammalian CNS. The depolarizing actions of GABA on central neurons have been known for some time (cf. Alger and Nicoll 1982; Kaila 1994; Kaila and Voipio 1987), but only recently has the truly excitatory aspect of GABA<sub>A</sub> receptor function in the adult hippocampus been recognized (Grover et al. 1993; Kaila et al. 1997; Staley et al. 1995; Taira et al. 1997). In rat hippocampal slice the excitatory actions of GABA become most evident on high-frequency stimulation of the interneuronal network. As we recently demonstrated in rat hippocampal pyramidal neurons, under such conditions the GABA<sub>A</sub> receptor–mediated depolarization and associated spike firing can far exceed that provided by the glutamatergic drive (Taira et al. 1997). The GABA-mediated depolarizing postsynaptic potentials (hereafter termed GDPSPs) that are evoked on high-frequency stimulation in hippocampal pyramidal neurons appear to be due to a network-driven, bicarbonate-dependent increase in extracellular K<sup>+</sup> ([K<sup>+</sup>]<sub>e</sub>)(Kaila et al. 1997; Lamsa and Kaila 1997).

In contrast to its conventional inhibitory role in the adult brain, during the early stages of postnatal development (up to postnatal day 8–10 in the rat), GABA may act as an excitatory transmitter in the CNS (Ben-Ari et al. 1989; Cherubini et al. 1991). Consistently with this, it has been shown that both synaptic and pharmacological stimulation of GABA<sub>A</sub> receptors leads to an elevation in intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) through activation of voltage-gated calcium channels (VGCCs) in pyramidal cells and interneurons in the immature rat hippocampus (Leinekugel et al. 1997), thus contributing to the neurotrophic actions of GABA during the neonatal period (Barbin et al. 1993). In adult hippocampal slices, synaptically induced transmembrane Ca<sup>2+</sup> shifts have almost exclusively been attributed to the stimulation of ionotropic glutamate receptors (see Heinemann et al. 1990), and most of the ensuing calcium uptake appears to be mediated by plasmalemmal VGCCs (Heinemann et al. 1990; Miyakawa et al. 1992; Paalasmaa and Kaila 1996). Because activation of the GABA<sub>A</sub> receptors can result in conspicuous depolarization and spike firing in the postsynaptic neuron, an obvious question to ask is whether GDPSPs are linked with a significant neuronal uptake of calcium. In the present study we provide compelling physiological and pharmacological evidence for this hypothesis.

Because stimulation-induced changes in the extracellular space (ECS) can affect local interstitial ion accumulations, in some of the experiments we also investigated the changes in the ECS volume paralleling tonic activation of the inhibitory network to better appreciate the magnitude of the accompanying Ca<sup>2+</sup> shifts.

Part of the results has appeared in an abstract form (Autere et al. 1997).

METHODS

Hippocampal slices (400 μm) from 30–40-day-old Wistar rats were prepared using established procedures (Taira et al. 1993). The experiments were carried out in an interface-type recording chamber (volume, 0.6 ml; flow rate, 1.0 ml/min; temperature 32°C), and the slices were allowed to recover for ~1 h before recording began.

The physiological solution contained (in mM) 124 NaCl, 3.0 KCl, 2.0 CaCl<sub>2</sub>, 25 NaHCO<sub>3</sub>, 1.1 NaH<sub>2</sub>PO<sub>4</sub>, 2.0 MgSO<sub>4</sub>, and 10 d-glucose.
It should be noted that in the bicarbonate-buffered solution the concentration of extracellularly available Ca²⁺ is 1.55–1.6 mM (see Heinemann et al. 1990). The solution was equilibrated with 95% O₂-5% CO₂ to yield a pH of 7.4. A stream of the same gas mixture was (following warming and humidifying) continuously passed over the preparation. In some of the experiments, NaHCO₃ was replaced by 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and the solution was gassed with 100% O₂. The pH was adjusted to 7.4 with NaOH. For the ECS volume measurements, 0.5 mM tetrabutylammonium ion (TMA⁺) was added into the perfusion solution.

Double-barreled microelectrodes were pulled from borosilicate glass (2GC150FS, Clark Electromedical, Pangbourne, Reading, UK). The nonfilamented barrel was silanized by exposure to vapor of dimethyl-trimethyl-silylamine (Fluka) followed by baking in an oven at 200°C. The following procedure was applied for Ca²⁺-selective microelectrodes. After dry beveling, the silanized barrel was back-filled with a solution containing 1 mM CaCl₂, 100 mM NaCl, and 1 mM HEPES (pH 7.6), and a short column of the Ca²⁺-selective ionophore (Fluka membrane cocktail 21048) was taken into the tip by suction. The reference barrel was filled with 150 mM NaCl. The outer diameter of these microelectrodes was 2–8 μm, and the resistances of the Ca²⁺ and reference barrels were 15–20 GΩ and 20–40 MΩ, respectively. The electrodes had a slope of 28–30 mV for a 10-fold change in [Ca²⁺]₀ and they were calibrated in terms of free concentration. Measurements of the extracellular concentration of bath-applied impermeable ions such as TMA⁺ can be used to study transient changes in the ECS volume (see Nicholson and Phillips 1981). In principle, changes in the ECS volume and in the TMA⁺ concentration are inversely proportional. TMA⁺-sensitive electrodes were manufactured in a manner similar to the Ca²⁺-electrodes except that the silanized barrel was filled with a solution containing (in mM) 150 NaCl, 3.5 KCl, and 0.5 TMA and the ionophore used was TMA⁺-selective (Corning 473717). The resistance of the TMA⁺-selective barrel was 0.5–1.0 GΩ, and the electrode had a slope of 55–59 mV per decade change.

All recordings were made in stratum radiatum of area CA1. The tip of the bipolar stimulus electrode was positioned close to (within 0.5 mm) the recording site. Stimulus intensity was supramaximal (10–30 V) and pulse duration 0.1 ms. High-frequency trains of pulses (100–200 Hz, 40–100 pulses) were given at 3-min intervals. Typically, after the first train the responses remained very stable (cf. Kaila et al. 1997).

RESULTS

We first investigated the GDPSP-linked changes in the ECS volume and in [Ca²⁺]₀ employing simultaneously TMA⁺ and Ca²⁺-selective microelectrodes. Upon high-frequency stimulation and in the presence of the ionotropic glutamate receptor antagonists, there was a 143 ± 14% (mean ± SE, n = 6) increase with time-to-peak 3.5 ± 0.9 s in the extracellular TMA⁺ concentration, thus indicating a corresponding decrease in the ECS volume (Fig. 1). Concomitantly, a 0.2–0.4 mM transient fall in [Ca²⁺]₀, which reached its peak amplitude in 1–1.5 s and a field potential transient consisting of 1–3 mV negative deflections with a time-to-peak ranging between 0.7 and 1.0 s were observed. If the shrinkage of the ECS volume at the time of the peak fall in [Ca²⁺]₀ is taken into account, the net loss of Ca²⁺ from the ECS was 15–35% higher. In further measurements of the ECS volume changes, we noted that application of the ionotropic glutamate receptor antagonists diminished the ECS shrinkage to 51 ± 11% (n = 3, not illustrated) of the control values. Thereafter, upon exposure to PiTX, a further 15 ± 4% (n = 3) attenuation in the ECS volume change was seen. This will correspondingly accentuate the drop in [Ca²⁺]₀, and therefore the shifts in the ECS volume cannot underlie the observed alleviation of the [Ca²⁺]₀ transients by the drug treatments.

Upon application of the GABA_A receptor antagonist PiTX (100 μM) in the absence of the ionotropic glutamate receptor blockers, there was first a decrease in the GDPSP-induced [Ca²⁺]₀ shift to 74 ± 2% of the control value (P < 0.05, paired t-test, n = 4; within 3–6 min from start of wash-in) and only subsequently did the [Ca²⁺]₀ response start to gain size (Fig. 2A). If the sole function of the GABA_A receptors was to suppress neuronal activity, one would expect that exposure to the receptor antagonist would result in an immediate increase in synaptically evoked [Ca²⁺]₀ shifts. This is probably true when stimulation paradigms not reaching the threshold for triggering GDPSPs are used (cf. Hamon and Heinemann 1986). However, the rather atypical nature of the PiTX effect in the present study is consistent with our recent observation of a similar biphasic effect of PiTX on postsynaptic spike firing evoked on high-frequency tetanus (Taira et al. 1997). Subse-
quent application of the ionotropic glutamate receptor antagonists AP5, NBQX, and ketamine effectively attenuated the \([\text{Ca}^{2+}]_0\) shift. Exposure to the glutamate blockers alone decreased the tetanus-evoked fall in \([\text{Ca}^{2+}]_0\) to 66 ± 3% of the control value \((P < 0.005, \text{paired } t\text{-test}, n = 9, \text{Figs. 2B and 3}).

Because we next wanted to study \([\text{Ca}^{2+}]_0\) transients evoked solely on synaptic activation of \(\text{GABA}_A\) receptors, all experiments described after this point were done in the continuous presence of the ionotropic glutamate receptor antagonists. Blockade of the \(\text{GABA}_A\) receptors by PiTX in the presence of the glutamate receptor antagonists diminished the \([\text{Ca}^{2+}]_0\) transient, preserving only 20–30% of the original shift \((P < 0.005, \text{t-test}, n = 8, \text{Figs. 4A and 6}). A similar result (48 ± 7% decrease in the \([\text{Ca}^{2+}]_0\) shift, \(P < 0.005, \text{t-test}, n = 3)\) was obtained by using a competitive \(\text{GABA}_A\) receptor antagonist, bicuculline \((10 \mu\text{M})\) thus further confirming the involvement of \(\text{GABA}_A\) receptors in the phenomenon (not illustrated).

To further elucidate the link between synaptically evoked \([\text{Ca}^{2+}]_0\) shifts and \(\text{GABA}_A\)-ergic transmission, we examined the effect of PB \((100 \mu\text{M})\), a widely used potentiator of the \(\text{GABA}_A\) receptor function, on the \([\text{Ca}^{2+}]_0\) transients. Application of PB resulted in a significant (126 ± 6%) increase \((P < 0.05, \text{paired } t\text{-test}, n = 4)\) in the peak fall in \([\text{Ca}^{2+}]_0\) (Figs. 4B and 6). The accentuation of the response was seen even more clearly as an increase in its duration (Fig. 4B).

To demonstrate the causal connection between depolarizing \(\text{GABA}_A\) receptor-mediated responses and the \([\text{Ca}^{2+}]_0\) transients, it was of importance to examine the \([\text{Ca}^{2+}]_0\) shifts under experimental conditions known to suppress GDPSPs. This was done by taking advantage of the known \(\text{HCO}_3\) dependency of the depolarizing \(\text{GABA}_A\) responses. GDPSPs can be attenuated upon application of membrane-permeant inhibitors of carbonic anhydrase \((\text{Grover et al. 1993; Staley et al. 1995; Taira et al.})\).

\[\text{\text{FIG.2.} A: biphasic effect of picrotoxin (PiTX; 100 \mu\text{M}) on stimulation-induced [Ca}^{2+}]_0\text{, transients in stratum radiatum in the absence of glutamate receptor antagonists. Note that the increase of the [Ca}^{2+}]_0\text{, transient corresponds to the appearance of early spiking. In this and the following figures, top traces are field potentials and bottom traces [Ca}^{2+}]_0\text{, recordings. B: effects of the glutamate antagonists \text{d'2-amino-5-phosphonopentoate (AP5; 40 \muM), 6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione (NBQX; 10 \muM), and ketamine (50 \muM) on stimulation-induced [Ca}^{2+}]_0\text{, transients in stratum radiatum. Despite complete block of ionotropic glutamate receptors, there is only a partial block of the [Ca}^{2+}]_0\text{, shift.}}\]
In the presence of the membrane-permeant carbonic anhydrase inhibitor EZA (50 μM), the \([\text{Ca}^2+]_o\) shift was diminished to 68 ± 2% (\(P < 0.005, t\)-test, \(n = 4\)), Figs. 5 and 6). A similar kind of result (diminution of \([\text{Ca}^2+]_o\) shift to 36 ± 6%, \(P < 0.005, t\)-test, \(n = 4\)) was achieved by replacing the perfusion solution by HEPES-buffered HCO\textsubscript{3}⁻-free solution (not illustrated).

DISCUSSION

Common knowledge holds that a strong GABA\textsubscript{A}-mediated input will result in effective shunting of the postsynaptic membrane, thus suppressing excitatory synaptic responses and attenuating the activation of the main Ca\textsuperscript{2+} conductive pathways, the VGCCs and \(N\)-methyl-\(D\)-aspartate (NMDA) receptor channels (for \([\text{Ca}^2+]_o\) responses, see Hamon and Heinemann 1986; Heinemann et al. 1984). Yet, it is becoming increasingly evident that GABAergic interneurons do not merely gate the activity of principal cells, but upon intense stimulation they can have an auxiliary or even dominating role in eliciting postsynaptic firing (see Taira et al. 1997). The rather unconventional idea of GABA\textsubscript{A} receptor-mediated excitation in the adult hippocampus has been established only recently (see introduction), and therefore the physiological consequences of this phenomenon are largely unknown. We focused our study to resolve the apparent but still unanswered question of whether GABAergic excitation might accentuate activity-dependent changes in \([\text{Ca}^2+]_o\) in the adult CNS.

**Synaptic mechanisms of \([\text{Ca}^2+]_o\) shifts evoked by high-frequency stimulation**

Even in the presence of PiTX, NMDA receptors contribute little to the stimulus-evoked transient fall in \([\text{Ca}^2+]_o\) in the area...
CA1 (Köhrl and Heinemann 1989; Paalasmaa et al. 1994), whereas under such conditions most of the \([\text{Ca}^{2+}]_o\) shift can be attributed to activation of non-NMDA glutamate receptors and a consequent triggering of VGCCs (Heinemann et al. 1990; Paalasmaa and Kaila 1996; Paalasmaa et al. 1994). Accordingly, upon tetanic stimulation of the Schaffer collaterals, Miyakawa et al. (1992) detected hardly any \(\text{Ca}^{2+}\) influx through the dendritic NMDA receptor channels using intracellularly injected fura-2. Hence they concluded that the observed rise in intracellular \(\text{Ca}^{2+}\) was mainly due to activation of VGCCs.

In the present study we found that only a part of the \([\text{Ca}^{2+}]_o\) shift evoked by stimuli applied at 100 or 200 Hz close to the site of recordings was blocked by the ionotropic glutamate receptor antagonists. The rather long duration of \([\text{Ca}^{2+}]_o\) transients seen in the absence and presence of the glutamate antagonists is congruent with the long duration of GDPSPs and the accompanied spike firing and increase in \([\text{K}^+]_o\) (cf. Kaila et al. 1997; Taira et al. 1997).

Application of PiTX under control conditions (before application of the glutamate receptor antagonists) resulted in an interesting sequence of events: first, a slight decrease in the \([\text{Ca}^{2+}]_o\) shift and, thereafter, an augmentation of the response. In previous studies employing relatively weak stimulation protocols or glutamate agonist application, the GABA\(_A\) receptor antagonists bicuculline and PiTX have been shown to monotonically enhance decreases in \([\text{Ca}^{2+}]_o\) (e.g., Hamon and Heinemann 1986). The biphasic effect of PiTX on \(\text{Ca}^{2+}\) shifts in the present study is apparently paralleled by changes in postsynaptic spike firing on high-frequency stimulation (Taira et al. 1997). Because the GDPSPs are more sensitive to GABA\(_A\) receptor antagonists than the hyperpolarizing inhibitory postsynaptic potentials (IPSPs) (cf. Alger and Nicoll 1982), PiTX will first attenuate the excitatory GABA responses (and overall excitation), and, only after continuous wash-in of the drug, the hyperpolarizing responses will be affected, thus resulting in an enhancement of the early spiking mediated by ionotropic glutamate receptors (Taira et al. 1997). Consequently, regarding the fact that most of the calcium influx seen on tetanic stimulation of afferents ensues from the activation of VGCCs, the biphasic effect of PiTX on \(\text{Ca}^{2+}\) transients is easy to understand. As expected, applying PiTX on top of the glutamate antagonists strongly attenuated the \(\text{Ca}^{2+}\) shift. To exclude the possibility that this finding was due to a nonspecific effect of PiTX on \(\text{Ca}^{2+}\) channels or Cl\(^-\) transport (cf. Gross et al. 1997; Kaila et al. 1997), we used bicuculline instead of PiTX in some of the experiments. The remaining component of the \(\text{Ca}^{2+}\) response seen in the presence of ionotropic glutamate and GABA\(_A\) receptor antagonists is suggested to arise from a presynaptic \(\text{Ca}^{2+}\) influx (see Heinemann et al. 1990) and/or direct stimulation of a small population of neurons (see Paalasmaa and Kaila 1996).

PB, which is known to potentiate the GABA\(_A\) receptor function is known to enhance depolarizing GABA responses (Alger and Nicoll 1982; Lamsa and Kaila 1997) and associated bicarbonate shifts (Kaila et al. 1992; Taira et al. 1995b; Voipo et al. 1995). In line with the idea that GABA-mediated depolarization can augment neuronal uptake of \(\text{Ca}^{2+}\), this drug potentiated the tetanus-evoked \(\text{Ca}^{2+}\) transients. This finding is again contradictory to the conventional view that agents enhancing GABAergic transmission will reduce transmembrane \(\text{Ca}^{2+}\) shifts in the adult brain.

Depolarizing GABA responses evoked by high-frequency stimulation are dependent on the availability of bicarbonate. Application of the membrane-permeant carbonic anhydrase inhibitor, EZA, or replacing the perfusion solution with nominally HCO\(_3^-\)/CO\(_2\)-free solution buffered with HEPES leads to a suppression of the network-driven GDPSPs (Kaila et al. 1997; Taira et al. 1997). Hence it was of much interest that both treatments also led to a mitigation of GABA-evoked \(\text{Ca}^{2+}\) shifts in the present experiments. In our recent study on the excitatory GABA responses in pyramidal neurons, a partial suppression only of posttetanic GABAergic depolarization was achieved by EZA, thus explaining the incomplete blockade of \(\text{Ca}^{2+}\) transients by the drug (cf. Fig. 3 in Taira et al. 1997). Evidently then, noncatalyzed (de)hydration of CO\(_2\) in the absence of carbonic anhydrase activity is able to produce bicarbonate in a sufficient amount for a large part of the GABA\(_A\) receptor-dependent interneuronal excitation and its ionic consequences to persist (Lamsa and Kaila 1997).

**Neuronal versus glial origin of the \(\text{Ca}^{2+}\) sink**

The cellular elements in the nervous tissue taking up \(\text{Ca}^{2+}\) on tetanic stimulation cannot, of course, be directly inferred from the data obtained using ion-selective extracellular microelectrodes. The following question then arises: what are the relative contributions of neurons and glial cells to the \([\text{Ca}^{2+}]_o\) changes seen in the present study. It is known that a robust increase in \([\text{K}^+]_o\) of the kind seen during some nonphysiological conditions such as ictal activity can trigger astroglial \(\text{Ca}^{2+}\) influx via VGCCs (Duffy and MacVicar 1994). However, activation of glial VGCCs requires large elevations (threshold 20–25 mM) in \([\text{K}^+]_o\) (Duffy and MacVicar 1994). As reported in our recent paper (Kaila et al. 1997), GDPSP-linked \([\text{K}^+]_o\) shifts are typically in the range of 7–9 mM. Moreover, under physiological conditions glial cells do not fire spikes, a property that underlies much of the neuronal uptake of \(\text{Ca}^{2+}\). Nevertheless, in the light of the existing data, it seems likely that under the present experimental conditions there is both neuronal as well as glial uptake of \(\text{Ca}^{2+}\), albeit the relative contributions of neuronal and glial \(\text{Ca}^{2+}\) sinks remain to be elucidated.

**Implications of the present findings**

As demonstrated in the present work, activation of GABA\(_A\) receptors can lead to prominent transmembrane calcium fluxes adding to the synaptic mechanisms promoting neuronal \(\text{Ca}^{2+}\) entry. Activation of a certain population of dendritic calcium channels, namely the low-voltage activated (LVA) channels is enhanced if it is preceded by hyperpolarization (Magee and Johnston 1995). Apparently, excitatory postsynaptic potentials (EPSPs) following hyperpolarizing IPSPs would particularly favor the contribution of LVA \(\text{Ca}^{2+}\) channels to postsynaptic \(\text{Ca}^{2+}\) entry. Therefore the sequential postsynaptic hyperpolarization/depolarization in pyramidal neurons resulting from intense stimulation of interneurons is especially well-suited for promoting the activation of LVA \(\text{Ca}^{2+}\) channels (Lambert and Grover 1995). This raises the interesting possibility that at least part of the elevation of postsynaptic \(\text{Ca}^{2+}\) needed for the induction of tetanus-induced forms of LTP results from the activation of interneurons (cf. Cavus and Teyler 1996; Grover and Teyler 1990; Taira et al. 1995a). The present results also suggest that GABA-mediated excitation might act as a “hand-shaking” signal (see Marty
and Llano 1995) to promote Ca\(^{2+}\)-dependent Hebbian-type plasticity changes at GABAergic synapses.

It was reported by Leinekugel et al. (1995) that synaptic activation of the GABA\(_A\) receptors leads to depolarization and rise in intracellular Ca\(^{2+}\) via activation of VGCCs in 2–5 day-old but not in 12–13 day-old rat hippocampal slices. Furthermore, in neonates the GABA\(_A\) receptor–mediated depolarization is essential for activity-induced plasticity of GABA\(_A\)-mediated transmission (McLean et al. 1996). However, whereas Leinekugel et al. (1995) used only low-frequency (4–5 Hz, 4–5 pulses) stimulation, we intentionally selected to employ high-frequency (100–200 Hz, 40–100 pulses) trains of pulses to achieve tonic activation of the interneuronal GABAergic network. Thus, depending on the stimulation/firing pattern of interneurons, a large fraction of the resulting Ca\(^{2+}\) transient in the area CA1 can be mediated by GABAergic originating in pyramidal neurons. Therefore the conclusion that synaptic activation of GABA\(_A\) receptors leads to Ca\(^{2+}\) uptake in immature but not in mature neurons may be premature.

This study was supported by grants from the Academy of Finland, the Sigrid Juselius Foundation, and the University of Helsinki.

Address for reprint requests: T. Taira, Dept. of Biosciences, Division of Animal Physiology, P.O. Box 17, University of Helsinki, FIN-00014 Helsinki, Finland.

Received 13 February 1998; accepted in final form 8 October 1998.

REFERENCES


