Physiological Properties of GABA <sub>A</sub> Receptors From Acutely Dissociated Rat Dentate Granule Cells

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Introduction

GABA mediates inhibitory neurotransmission in the forebrain by activation of GABA<sub>A</sub> and GABA<sub>B</sub> receptors. Study of fast, GABA<sub>A</sub> receptor-mediated, inhibitory postsynaptic currents (IPSCs) in hippocampal dentate granule cells has suggested that properties of GABA<sub>A</sub> receptors influence the amplitude and time course of the IPSCs. This study describes the physiological properties of GABA<sub>A</sub> receptors present on hippocampal dentate granule cells acutely isolated from 18- to 35-day-old rats. Rapid application of 1 mM GABA to outside-out macropatches excised from granule cells produced GABA<sub>A</sub> receptor currents with rapid rise time and biexponential decay of current after removal of GABA. After activation, granule cell GABA<sub>A</sub> receptor currents desensitized incompletely. During a 400-ms application of 1 mM GABA, peak current only desensitized ~40%. In symmetrical chloride solutions there was no outward rectification of whole cell current. Activation rates and peak currents elicited by rapid application of GABA to macropatches were also similar at positive and negative holding potentials. However, deactivation of GABA<sub>A</sub> receptor currents was slower at positive holding potentials. When whole cell currents were recorded without ATP in the pipette, current run-down was not apparent for 30 min in 50% of neurons, but run-down appeared to start soon after access was established in the remaining neurons. When 2 mM ATP was included in the recording pipette no run-down was apparent 30 min of recording. The efficacy and potency of GABA were lower in cells recorded with no ATP in the pipette and during run-down compared with those recorded with 2 mM ATP and no run-down.

Methods

Isolation of dentate granule cells

All experiments were performed on dentate granule cells isolated according to the method described originally by Kay and Wong (1986). Male or female Sprague-Dawley rats (18–35 days old) were euthanized and decapitated. The brain was dissected free, and the region containing the hippocampus was blocked and chilled in an oxygenated PIPES-buffered medium (4°C) for 1 min. The PIPES buffer solution contained (in mM) 120 NaCl, 2.5 KCl, 1.5 CaCl<sub>2</sub>, 1
MgCl₂, 25 mM-glucose, and 20 PIPES (pH 7.0). After blot drying, the brain was mounted on a vibratome stage, and 500-μm coronal sections containing the hippocampus were cut. The sections were allowed to recover in oxygenated (95% O₂-5% CO₂) PIPES-buffered medium in fire-polished glass pipettes to isolate neurons. The isolated neurons were plated on poly-l-lysine-coated 35-mm polystyrene Petri dishes (Corning Glass Works; Corning, NY), and the recordings were made within 1 h of isolation.

Recording and analysis of GABA<sub>A</sub> receptor currents from outside-out macropatches excised from acutely isolated dentate granule cells

Patch-clamp recordings were performed on outside-out membrane patches (Hamill et al. 1981) pulled from acutely isolated dentate granule cells bathed in an external solution consisting of (in mM) 142 NaCl, 8 CsCl, 6 MgCl₂, 1 CaCl₂, 10 HEPES, and 10 glucose (pH 7.4, 330 mosmol) at room temperature. Intrappetite CsCl was used to block potassium currents. Glass microelectrodes were formed from thick-walled borosilicate glass (World Precision Instruments) with a Flaming Brown electrode puller, fire polished to tip resistances of 10–20 MΩ, and then coated with Q-dope. Patch electrodes were filled with an internal solution consisting of (in mM) 153 CsCl, 1 MgCl₂, 2 MgATP, 10 HEPES, and 5 EGTA (pH 7.3, 300 mosmol). This combination of internal and external solutions produced a chloride equilibrium potential of 0 mV. Outside-out membrane patches were voltage clamped at −50 mV with an EPC-7 amplifier (List; Darmstadt, Germany).

GABA was applied to outside-out membrane patches with a rapid application system (Franke et al. 1987) consisting of a double-barreled theta tube (FHC, Brunswick, ME) connected to a piezoelectric translator (Burleigh Instruments; Fishers, NY). One barrel was perfused with the external recording solution, and the other was perfused with a GABA-containing external solution. Activation of the translator drove the solution interface rapidly across the patch surface. The solution exchange time was routinely monitored at the end of each recording by blowing out the patch and stepping a dilute (90%) external solution across the open electrode tip to measure a liquid junction potential; 10–90% rise times for solution exchange were consistently <400 μs. The recording chamber was continuously perfused with external solution to prevent accumulation of GABA in the bath. All experiments were performed at room temperature (22–23°C).

Outside-out patch data were low-pass filtered at 3 kHz, digitized at 10 kHz, and analyzed with the pClamp6 software suite (Axon Instruments; Foster City, CA) and Origin 4.1 (Microcal; Northampton, MA). Multiple GABA-elicited responses (5–20) were acquired for each patch at 30-s intervals and then were averaged to form ensemble currents for analysis. Activation of ensemble currents was measured as a 10–90% rise time to the peak current. The desensitization or deactivation time courses of ensemble GABA<sub>A</sub> receptor currents were fit with the Levenberg-Marquardt least-squares method with one or two component exponential functions. The number of exponential components was determined by statistically comparing the sum of squared residuals for one and two component fits (F test, P < 0.001).

The extent of desensitization was measured as (peak current − fitted steady state current)/peak current). Numerical data were expressed as means ± SE. Statistical significance was determined with paired two-tailed t-tests (P < 0.05).

**RESULTS**

**Activation and deactivation of dentate granule cell GABA<sub>A</sub> receptor outside-out patch currents**

In the synaptic cleft of central GABAergic synapses, the GABA concentration has been estimated to be high (0.5–1 mM) after release of GABA from presynaptic terminals (Edwards et al. 1990; Jones and Westbrook 1995; Maconochie et al. 1994). To mimic the time course of GABA in the synaptic cleft, 1 mM GABA was applied briefly to outside-out membrane patches excised from acutely isolated dentate granule cells with a piezoelectric translator to make rapid switches between solutions. GABA was applied to individual patches every 20–30 s during each experiment. Open-tip currents were routinely checked at the end of experiments and indicated a
rapid solution exchange time under these experimental conditions (10–90% rise time 360 ± 120 μs, n = 6).

Transient (2 ms) application of 1 mM GABA to outside-out membrane patches voltage clamped to −50 mV produced rapidly activating and deactivating currents (Fig. 1). In the example shown, the current activated with a 10–90% rise time of 0.90 ms (Fig. 1A) and deactivated with a biexponential time course with time constants of 9.7 (58.1%) and 100 ms (41.9%) (Fig. 1B). The average rise time of the outside-out patch GABA_A receptor currents, although rapid (10–90% rise time, 0.90 ± 0.09 ms, n = 6), was two to three times slower than the solution exchange time. This rise time was similar to reported dentate granule cell IPSC rise times (Edwards et al. 1990) but was clearly slower than that reported by De Koninck and Mody (1994).

Deactivation of dentate granule cell GABA_A receptor currents was studied after brief (2 ms), rapid application of 1 mM GABA for 400 ms to outside-out patches excised from acutely isolated dentate granule cells. In the example shown, the current desensitized with two exponential components with time constants (and relative proportions) of 12.4 ms (26.0%) and 218 ms (74.0%) (Fig. 2). During the 400-ms period of application, average GABA_A receptor currents desensitized with a biexponential time course to 60 ± 4.9% of the peak response (n = 7). The fast phase of desensitization (τ_fast 16.4 ± 2.7 ms) contributed to 76% of the total desensitization, whereas the slow phase (τ_slow 211 ± 31.6 ms) contributed the remainder of the desensitization (Table 1).

**Table 1. Rapid kinetic properties of dentate granule cell GABA_A receptors**

<table>
<thead>
<tr>
<th>Activation, 2 ms</th>
<th>n</th>
<th>10–90% rise time, ms</th>
<th>0.90 ± 0.09</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deactivation, 2 ms</td>
<td>n</td>
<td>τ_fast, ms</td>
<td>9.9 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>τ_slow, ms</td>
<td>95.8 ± 10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent fast</td>
<td>63.1 ± 2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent slow</td>
<td>36.9 ± 2.3</td>
</tr>
<tr>
<td>Activation, 400 ms</td>
<td>n</td>
<td>10–90% rise time, ms</td>
<td>0.72 ± 0.09</td>
</tr>
<tr>
<td>Desensitization, 400 ms</td>
<td>n</td>
<td>τ_fast, ms</td>
<td>16.4 ± 2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>τ_slow, ms</td>
<td>211 ± 31.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent fast</td>
<td>39.4 ± 9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Percent slow</td>
<td>60.6 ± 9.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Extent, %</td>
<td>39.7 ± 4.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n is number of cells studied.

**Desensitization of dentate granule cell GABA_A receptor outside-out patch currents**

Desensitization of GABA_A receptor currents was studied after rapid application of 1 mM GABA for 400 ms to outside-out patches excised from acutely isolated dentate granule cells. In the example shown, the current desensitized with two exponential components with time constants (and relative proportions) of 12.4 ms (26.0%) and 218 ms (74.0%) (Fig. 2). During the 400-ms period of application, average GABA_A receptor currents desensitized with a biexponential time course to 60 ± 4.9% of the peak response (n = 7). The fast phase of desensitization (τ_fast 16.4 ± 2.7 ms) contributed to 76% of the total desensitization, whereas the slow phase (τ_slow 211 ± 31.6 ms) contributed the remainder of the desensitization (Table 1).

**Voltage dependence of whole cell dentate granule cell GABA_A receptor currents**

The voltage dependence of dentate granule cell GABA_A receptor currents was studied with symmetrical transmembrane chloride concentration. Under these conditions, reversal of the currents occurred at 0 mV, and identical amplitude outward and inward currents were recorded in response to 10 μM GABA applied to cells voltage clamped to +50 mV and −50 mV (Fig. 3A). The current–voltage relationship of dentate granule cell GABA_A receptor currents elicited by 10 μM GABA with symmetrical transmembrane chloride concentrations was linear (n = 5 neurons) (Fig. 3B).

**Fig. 1.** Biphasic deactivation of an outside-out membrane patch current after a 2-ms application of 1 mM GABA. An outside-out membrane patch pulled from an acutely isolated dentate granule cell was voltage clamped at −50 mV and rapidly exposed to 1 mM GABA for 2 ms. A: current activated rapidly with a 10–90% rise time of 0.90 ms. B: current deactivated with a biphasic time course (τ_f = 9.7 ms, τ_s = 100 ms) with 58.1% of the fitted decay in the fast component.

**Fig. 2.** Biphasic desensitization of an outside-out membrane patch current after 400-ms application of 1 mM GABA. An outside-out membrane patch pulled from an acutely isolated dentate granule cell was voltage clamped at −50 mV and rapidly exposed to 1 mM GABA for 400 ms. The current desensitized with a biphasic time course (τ_f = 12.4 ms, τ_s = 218 ms) with 26.0% of the fitted desensitization in the fast component. The extent of desensitization after 400 ms was 56.7%.
Voltage dependence of dentate granule cell GABA<sub>A</sub> receptor outside-out patch currents

The effects of transmembrane potential on rectification, activation, and deactivation of GABA<sub>A</sub> receptor currents in outside-out patches were determined. GABA (1 mM) was rapidly applied for 2 ms to outside-out patches excised from acutely isolated dentate granule cells when the same membrane patch was voltage clamped to −50 and +50 mV (Fig. 4). The +50/−50-mV peak current ratio of 1.05 ± 0.11:1 (n = 5) demonstrated no difference in the peak currents at +50 and −50 mV. Although peak current amplitudes were identical at positive and negative holding potentials, the currents deactivated at different rates when patches were voltage clamped to +50 mV or to −50 mV. In the example shown, the outside-out patch currents deactivated with two similar time constants at both positive (τ<sub>f</sub> = 11.3 ms and τ<sub>s</sub> = 85.7 ms) and negative (τ<sub>f</sub> = 12.4 ms and τ<sub>s</sub> = 103.4 ms) holding potentials (Fig. 4A). However, at +50 mV current deactivation occurred equally with both components (α<sub>f</sub> = 51.2% and α<sub>s</sub> = 48.8%), whereas at −50 mV current deactivation occurred primarily with the fast component (α<sub>f</sub> = 72.9% and α<sub>s</sub> = 27.1%). The net result was that the current deactivated more rapidly at −50 mV (average deactivation time constant of 47.6 ms) than at +50 mV (average deactivation time constant of 36.3 ms at −50 mV). On average (n = 5 patches), when outside-out patches were clamped to −50 mV, the fast phase of deactivation occurred with a time constant 10.1 ± 1.9 ms and contributed 66 ± 3.9% of total desensitization. When patches were clamped to +50 mV the fast phase of deactivation was 6.9 ± 1.2 ms but only contributed 47 ± 6.2% of total deactivation. When patches were clamped to −50 mV, the slow phase of deactivation occurred with a time constant of 88.7 ± 13.6 ms and contributed 34 ± 3.9% of total deactivation, whereas when patches were clamped to +50 mV the slow phase of deactivation occurred with a time constant of 73.9 ± ms and contributed 56.0 ± 8.0% of total deactivation. The weighted sum of deactivation time constants when macropatches were voltage clamped to −50 mV was 35.9 ± 6.8 ms and at +50 mV was 42.8 ± 6.8 ms (P < 0.05, paired t-test) (Fig. 4B).

**ATP dependence of currents**

We determined the ATP dependence of dentate granule cell GABA<sub>A</sub> receptor currents. When whole cell GABA<sub>A</sub> receptor currents were recorded from acutely isolated dentate granule cells with pipettes that did not contain ATP (n = 8), two response patterns were noted. In one-half of the neurons studied, stable GABA<sub>A</sub> receptor currents in response to repeated application of 50 μM GABA were recorded for ≤30 min (Figs. 5, top panel, and 6, A), whereas in the remaining neurons GABA<sub>A</sub> receptor current began to decline (run-down) within 10 min of establishing access and continued to so steadily thereafter (Figs. 5, bottom panel, and 6, C). There were no obvious morphological or physiological differences between the neurons in which currents ran down and those they did not. When 2 mM ATP was included in the recording pipette, steady...
GABA_A receptor currents were recorded for 30 min \((n = 7)\), (Fig. 6, A).

To determine if the run-down in GABA_A receptor currents during the recording period with recording pipettes that did not contain ATP resulted from a reduction in the affinity and/or efficacy of GABA for GABA_A receptors, two GABA concentration–response relationships were determined in a single neuron before (Fig. 7A, top traces) and after (Fig. 7A, bottom traces) the onset of run-down. In this dentate granule cell, run-down resulted in a decrease in maximal GABA_A receptor current and increase in EC_{50} (Fig. 7B). However, because it took ~20 min to complete a concentration–response relationship, this experiment could not be repeated a sufficient number of times to quantify the effect. Therefore to determine the effect of run-down in GABA_A receptor an alternate approach was used. GABA concentration-GABA_A receptor–current relationships were determined in 13 dentate granule cells recorded with 2 mM ATP in the pipette (Fig. 8A) and in another 9 cells were recorded without ATP (Fig. 8B). In the neurons recorded with ATP in the pipette (Fig. 8A), the concentration–response data were recorded after two currents evoked by 10 μM GABA were identical. In neurons recorded without ATP in the pipette (Fig. 8B), if there was an initial increase in current data collection was started after two applications of 10 μM GABA elicited identical currents. Data collection was started immediately if run-down was apparent. Because run-down occurred as the concentration–response relationship was being studied, application of GABA in ascending concentrations might have resulted in the currents elicited by high GABA concentration that were smaller than if they had been recorded earlier. To compensate for this potential run-down, GABA was applied in ascending or descending concentration steps ranging from 1 to 1,000 μM in alternate neurons. Compared with granule cells recorded with 2 mM ATP, in granule cells recorded without ATP the threshold for evoking GABA_A receptor currents was higher, and smaller peak GABA_A receptor currents were recorded at all GABA concentrations (Fig. 8, A and B).

In dentate granule cells recorded with ATP in the pipette, the peak currents obtained in response to GABA concentrations ranging from 1 to 1,000 μM were fitted to a sigmoid function, and the equation for the best fit was used to derive EC_{50} and maximal GABA_A receptor currents elicited by GABA (Fig. 9, A). The mean EC_{50} for GABA for these neurons was 39 ± 6 μM, and the maximal current was 961 ± 102 pA \((n = 13)\). In dentate granule cells recorded without ATP in the pipette, the mean GABA EC_{50} was 117 ± 30 μM, and the maximal current was 377 ± 115 pA \((n = 9)\). The EC_{50} \((P < 0.05)\) was significantly higher in neurons recorded with no ATP in the pipette compared with those recorded with 2 mM ATP in the pipette. Maximal currents \((P < 0.05)\) were significantly smaller when obtained with no ATP in the recording in the pipette compared with those obtained with 2 mM ATP in the recording pipette. These findings were similar to the changes in GABA EC_{50} and maximal current caused by run-down observed in a neuron recorded with no ATP in the pipette.

**Discussion**

**Activation and deactivation properties**

Current models of GABAergic synapses terminating on the somata of dentate granule cells postulate that mIPSCs result from saturation of a small number of postsynaptic receptors by a high concentration of GABA (Busch and Sakmann 1990; De Koninck and Mody 1994; Edwards et al. 1990). The rapid rise time of mIPSCs on dentate granule cell somata was only partially replicated by application of 1 mM GABA for 2 ms to macropatches containing small numbers of receptors because the 10–90% rise time of GABA_A receptor activation current was substantially slower than the 290-μs rise time reported for mIPSCs (De Koninck and Mody 1994). One explanation of faster rise time mIPSCs on soma and proximal dendrites of dentate granule cells compared with the activation rate of GABA_A receptors on macropatches was that our application system was not sufficiently fast to replicate conditions in the synaptic cleft and test the saturation hypothesis. Alternatively, rapid rise times of mIPSCs are possible even for nonsaturating GABA concentrations if the time course of GABA in the synaptic cleft is rate limiting (Galarreta and Hestrin 1997). The...
deactivation of GABA<sub>A</sub> receptor currents on removal of saturating concentration of GABA was quantitatively and qualitatively distinct from the decay of mIPSCs recorded from the somata of dentate granule cells. Edwards et al. (1990) fitted the decay of mIPSCs with the sum of two exponential functions, with time constants of 2.0 ± 0.38 ms and 54 ± 18 ms. Several other studies (De Koninck and Mody 1994; Otis and Mody 1992; Soltesz et al. 1995) described the decay of mIPSCs by a single exponential function with a time constant of 3.7–7.2 ms. The deactivation of GABA<sub>A</sub> receptor currents from macropatches excised from dentate granule cells was fitted best to two exponential functions with time constants of 9.9 ± 1.8 and 95.8 ± 4.5 ms. There are several possible explanations for the discrepancy between the deactivation of macropatch GABA<sub>A</sub> receptor currents and mIPSC decay. In addition to the deactivation kinetic properties of the GABA<sub>A</sub> receptors, the mIPSC decay is likely to be effected by cable filtering of dendritic mIPSCs (Soltesz et al. 1995), rate of reuptake of GABA in the synaptic cleft, and unbinding and rebinding of GABA to the postsynaptic receptors. Additionally, we determined the deactivation properties in outside-out membrane patches pulled from the somata of dentate granule cells that may have included synaptic and extrasynaptic GABA<sub>A</sub> receptors; in contrast mIPSC decay largely depends on the properties of subsynaptic receptors.

Desensitization and rectification

Compared with CA1 pyramidal neuron GABA<sub>A</sub> receptor currents, dentate granule cell GABA<sub>A</sub> receptor currents had a slow desensitization rate and large residual current (Celentano and Wong 1994; Jones and Westbrook 1995). One possible explanation for this slow rate of desensitization may have been the presence of a δ subunit in these receptors. The mRNA for the δ subunit is well expressed in dentate granule cells, and δ subunit incorporation into recombinant GABA<sub>A</sub> receptors was shown to slow desensitization of the receptor currents (Fisher and Macdonald 1997; Haas and Macdonald 1998; Saxena and Macdonald 1994). Other possibilities include the potential con-
mIPSC decay in dentate granule cells (Otis and Mody 1992). With 2 nM ATP in the pipette (n = 13, ■) and recorded with no ATP in the pipette (n = 9, ●). Each point represented the mean of peak currents, and error bars showed SEs. The line was the best fit of data to a sigmoid function. The maximal current (I_{max}) and EC_{50} were derived from the equation for the sigmoid function that best fit the data. Note lower efficacy and potency of GABA when granule cells were recorded with no ATP in the pipette.

distribution of α4 subtype-containing isoforms to the currents and the modulation of desensitization kinetics by phosphorylation.

Dentate granule cell GABA_A receptor currents did not rectify at positive holding potentials when intracellular and extracellular chloride concentrations were symmetrical. There was a linear current–voltage relationship for unitary mIPSCs recorded from the somata of dentate granule cells (De Koninck and Mody 1994). In contrast outward rectification of GABA_A receptor single channel currents present on cultured hippocampal neurons (Segal and Barker 1984), hippocampal neurons in slices (Gray and Johnston 1985), and whole cell currents recorded from acutely isolated CA1 neurons (Burgard et al. 1996) have been reported. Burgard et al. (1996) demonstrated that the degree of rectification depended on the subunit composition of GABA_A receptors; for example, α1β3γ2 receptor currents rectified far less than α5β3γ2 receptor currents. It was likely that the linear current–voltage relationship of the dentate granule cell GABA_A receptor currents was a consequence of its distinct subunit composition.

The deactivation of GABA_A receptor currents from macro-patches after brief, rapid application was slower at +50 mV than at −50 mV. This was primarily due to greater participation of the slower phase of deactivation at +50 mV compared with −50 mV. This might explain the voltage dependence of mIPSC decay in dentate granule cells (Otis and Mody 1992). Slower deactivation of GABA_A receptor currents at depolarized potentials was described in cultured cerebellar granule cells (Mellor and Randall 1998).

Run-down of dentate granule cell GABA_A receptor current

Progressive loss or run-down of GABA_A receptor currents under whole cell recording conditions has been reported in many types of neurons, including acutely isolated CA1 pyramidal neurons (Stelzer et al. 1988), cultured chick spinal cord neurons (Gyenes et al. 1994), and recombinant receptors expressed in fibroblasts (Lin et al. 1994). Run-down of currents became apparent within 10 min in native neurons, and a large reduction of current occurred within 30 min. In contrast, in acutely isolated dentate granule cells in the absence of ATP in the recording pipette, either no run-down occurred for 30 min or run-down started in first 10 min of recording and then proceeded slowly. Clearly run-down and ATP dependence of acutely dissociated dentate granule cells and CA1 neurons was different where GABA_A receptor currents ran down rapidly in the absence of ATP in the recording pipette (Chen et al. 1990). The greater susceptibility of GABA_A receptor currents to run-down in acutely dissociated CA1 pyramidal neurons may have been due to their larger volume and greater metabolic needs, greater susceptibility to trauma associated with dissociation, or different GABA_A receptor isoform expressed by these cells.

This study suggested that the reduction in GABA_A receptor currents observed (when recorded with no ATP in the pipette) was due to reductions in both potency and efficacy of GABA. However this conclusion was limited because the current data were obtained during the development of run-down. Additionally, because the concentration–response curve recorded within the pipettes containing no ATP was not generated with sufficiently high GABA concentration to obtain a plateau current amplitude, the reported maximal current was likely underestimated, and a large reduction in GABA potency may have falsely appeared as a reduction in GABA efficacy. In another study a reduction in efficacy but increased potency of GABA in cultured chick neuron GABA_A receptors was reported (Gyenes et al. 1994). This probably reflected differences in isoforms or neurons used for the study.

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