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γ-Aminobutyrate, α-carboxy-2-nitrobenzyl ester selectively blocks inhibitory synaptic transmission in rat dentate gyrus

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Abstract

γ-Aminobutyrate, α-carboxy-2-nitrobenzyl ester (cGABA) is a stable photoactivatable probe used to study γ-aminobutyrate (GABA) receptors. GABA is released from this compound when it is exposed to ultraviolet light, but little is known about the electrophysiological effects of the compound itself. Whole cell patch clamp recordings on rat hippocampal slices demonstrated that cGABA blocked polysynaptic inhibitory postsynaptic currents (IPSCs) evoked in dentate granule cells by antidromic stimulation of the mossy fibers. It also reduced monosynaptically evoked IPSCs with an IC_{50} of 28 μM. In contrast, cGABA had no effect on excitatory postsynaptic currents (EPSCs) evoked by perforant path stimulation. The effect of cGABA was not mediated by depression of GABA release through activation of presynaptic $GABA_B$ receptors. cGABA inhibited muscimol-evoked currents by only 15% at a concentration of 40 μM. At this same concentration, it reduced the mean frequency of miniature inhibitory postsynaptic potentials by 71%, their mean peak amplitude by 44%, their mean decay time constant by 26% and the mean charge transfer per event by 52%. These effects may be explained by a phenothiazine-like modification of $GABA_A$ receptor kinetics and/or a selective block of somatic GABA synapses.

Keywords: GABA (γ-aminobutyrate); Caged compound; Synaptic inhibition; Hippocampus; Granule cell; $GABA_A$ receptor

1. Introduction

Laser-evoked photolysis of caged compounds is a novel method of administering neuroactive substances for experimental purposes. The active substance is released from an inactive precursor by a highly focused pulse of ultraviolet light (Katz and Dalva, 1994; Wang and Augustine, 1995; Nerbonne, 1996). The ''uncaging'' approach offers several advantages compared to conventional techniques, most notably a potentially high degree of spatial and temporal resolution. However, caged compounds must be carefully designed to avoid unwanted effects. Caged ATP, for example, was considered inert until it was discovered to activate ATP-dependent K^+ channels (Nichols et al., 1990). Some caged precursors of α_1-adrenoceptor agonists also have ''side-effects'', that is, they block voltage-gated Ca^{2+} channels (Nerbonne, 1996).

γ-Aminobutyrate (GABA) is the major inhibitory neurotransmitter in the central nervous system (Mott and Lewis, 1994; Johnston, 1996; Möhler et al., 1996). Selective modulation of GABA mechanisms has extraordinary significance from a pharmacotherapeutic point of view, because it influences the balance between excitation and inhibition. In animal models of temporal lobe epilepsy, the expression and pharmacological properties of $GABA_A$ receptors on dentate granule cells of the hippocampus were reported to be altered in a direction that might enhance the excitability of those neurons (Buhl et al., 1996; Gibbs et al., 1997; Brooks-Kayal et al., 1998; Shumate et al., 1998). Because such receptor changes might predispose the animals to seizures, we undertook investigations of this issue with use of the pilocarpine model. For this purpose, we made use of γ-aminobutyrate, α-carboxy-2-nitrobenzyl ester (cGABA), a compound from which GABA is released by exposure to ultraviolet light. cGABA was reported to possess almost ideal characteristics for a caged neurotrans-

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mitter precursor, including rapid photolysis and very slow spontaneous breakdown (Gee et al., 1994, 1998). Moreover, cGABA was reported to be biologically inactive. At concentrations as high as 500 μM, it did not induce $GABA_A$ receptor-mediated currents, and it did not antagonize the postsynaptic action of applied GABA in mouse cortical cultures (Gee et al., 1994). However, our preliminary experiments demonstrated that cGABA blocks inhibitory synaptic transmission in rat hippocampal slices. We then studied the effects of cGABA on pre- and postsynaptic GABA mechanisms and found a profile of actions that appears unique among GABA analogues.

2. Materials and methods

2.1. Preparation of hippocampal slices

Most experiments utilized previously untreated 100–125-g male Sprague–Dawley rats (Zivic-Miller Laboratories, Allison Park, PA, USA). Rats were decapitated under ether anesthesia, the brain was removed and 400-μm-thick transverse slices were cut from the caudal third of the hippocampal formation with a vibratome. The slices were transferred to a beaker that contained artificial cerebrospinal fluid (in mM: 122 NaCl, 25 $NaHCO_3$, 3.1 KCl, 1.8 $CaCl_2$, 1.2 $MgSO_4$, 0.4 KH_2PO_4 and 10 D-glucose, pH 7.4) and oxygenated at room temperature for at least 1.5 h with 95% O_2/5% CO_2.

Experiments that involved antidromic stimulation of the mossy fibers utilized rats that had been made epileptic by administration of pilocarpine, as previously described (Okazaki et al., 1999). Hippocampal slices were prepared at least 10 weeks after pilocarpine-induced status epilepticus. By that time, these animals had all developed an extensive network of recurrent excitatory connections among dentate granule cells. Thus, antidromic stimulation of granule cell axons (mossy fibers) evoked both a monosynaptic excitatory postsynaptic current (EPSC) and a polysynaptic inhibitory postsynaptic current (IPSC) (Okazaki et al., 1999).

2.2. Evoked synaptic responses

A slice was transferred to a glass-bottom submersion-type recording chamber mounted on the stage of a Nikon Optiphot-2 upright microscope (Nikon, Melville, NY, USA) connected to a Noran Odyssey confocal imaging system (Noran Instruments, Middleton, WI, USA). Artificial cerebrospinal fluid (10 ml) was recirculated at a rate of 4 ml/min at room temperature (22–24°C). Patch electrodes (5–7 MΩ) were pulled from borosilicate glass (1.5 mm outer diameter, 1.1 mm inner diameter, Sutter Instruments, Novato, CA) and were filled by vacuum with dipper solution (in mM: 140 cesium gluconate, 15 HEPES, 3.1 $MgCl_2$, 1 $CaCl_2$, 11 EGTA, pH 7.2, 276 mosM). Electrodes were then backfilled with internal solution that contained (in mM): 120 cesium gluconate, 10 HEPES, 2 MgATP, 1 EGTA, 5 creatine phosphate, 20 units/ml creatine phosphokinase, 10 *N*-ethyl lidocaine (QX-314) chloride, pH 7.2, 276 mosM. E_{Cl}, under these conditions, was about −50 mV. An Axopatch ID amplifier (Axon Instruments, Foster City, CA, USA) was used for whole cell patch clamp recording. Recordings were made with use of the ''blind'' approach (Blanton et al., 1989) from granule cells located in the infrapyramidal blade of the dentate gyrus, unless otherwise indicated. Cells with a membrane potential less negative than −70 mV upon break-in (after correction for a 10-mV liquid junction potential) were omitted from this study. Signals were filtered at 2 kHz, digitized at 10 kHz and stored to disk with use of a TL 1–125 digitizing board and pClamp6 (Axon Instruments) software. Series resistance (6–25 MΩ) was compensated ~50%. Activation of postsynaptic $GABA_B$ receptors was prevented by use of a cesium-based internal solution that included QX-314, but not GTP. Experimentation started at least 30 min after achieving whole cell access.

To evoke a monosynaptic IPSC, a bipolar stimulating electrode (25-μm-diameter nichrome wires, tip separation of 0.3 mm) was placed in the dentate molecular layer 200–500 μm from the recorded cell. Rectangular current pulses of 100 μs duration were applied every 30 s, and the stimulus current (200–300 μA) was adjusted to evoke a nearly maximal outward synaptic current recorded at a holding potential of 0 mV. Excitatory synaptic transmission was blocked with a combination of 5 μM 2,3-dihydroxy-6-nitro-7-sulfamyl-benzo(*F*)quinoxaline (NBQX) and 50 μM D-2-amino-5-phosphonopentanoate (D-AP5). The IC_{50} for the action of cGABA was computed with use of Prism3 (GraphPad Software, San Diego, CA, USA).

To evoke a polysynaptic feedback IPSC and a recurrent mossy fiber EPSC, the stimulating electrode was placed in stratum lucidum of area CA3b ~100 μm outside the dentate hilus. A field electrode was used to probe for the location in the granule cell body layer where the antidromic population spike was of greatest amplitude. After optimizing the position of the stimulating electrode along the *Z* axis, a whole cell patch clamp recording was established close to the field electrode. Stimulus intensity (300–500 μA) was adjusted to evoke a nearly maximal outward synaptic current at 0 mV holding potential. This current mainly represents the antidromically evoked polysynaptic IPSC. The inward current recorded at a holding potential of −80 mV mainly represents the recurrent mossy fiber EPSC (Okazaki et al., 1999).

To record monosynaptic excitatory responses, the stimulating electrode was placed in the perforant path where it crosses the subiculum. The superfusion medium contained 30 μM bicuculline methiodide to prevent contamination of the EPSC by feedforward inhibition. Recordings were made at a holding potential of −80 mV, and the stimulus

intensity (300–500 μA) was adjusted to evoke a nearly maximal response.

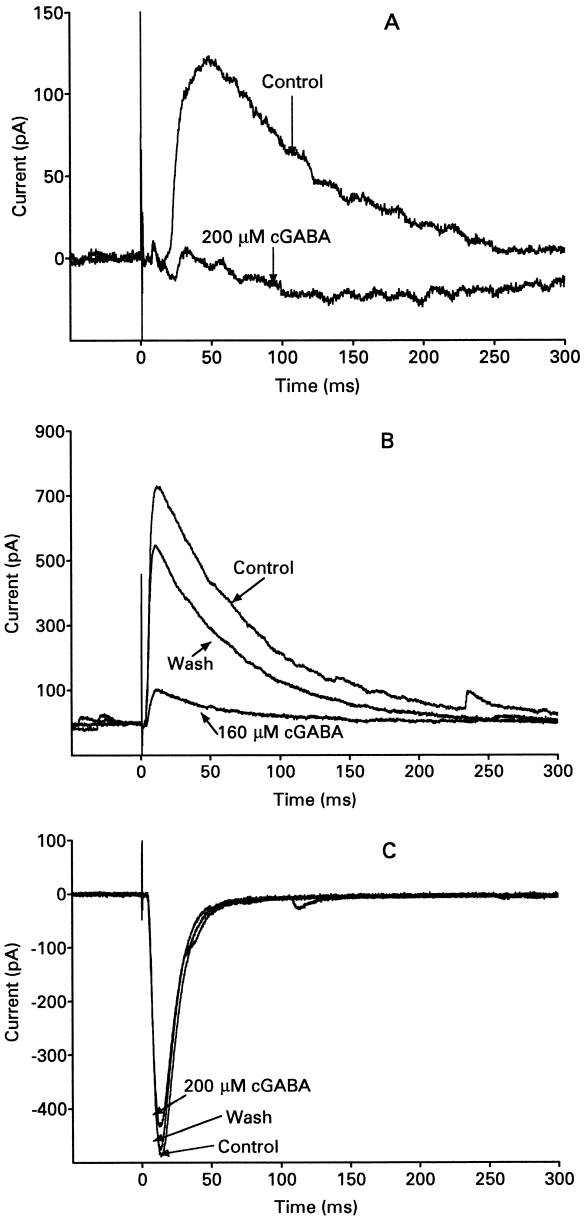

Fig. 1. Representative recordings that illustrate the effects of cGABA on inhibitory and excitatory synaptic transmission. Whole cell patch clamp recordings were made from dentate granule cells in rat hippocampal slices at room temperature. The stimulus was delivered at time 0. (A) cGABA (200 μM) almost completely eliminated the outwardly directed component of the current evoked by antidromic stimulation of the mossy fibers. Holding potential of 0 mV. The outward current recorded under these conditions represents the polysynaptically driven feedback IPSC. (B) cGABA (160 μM) markedly depressed the monosynaptic IPSC evoked by stimulation of the dentate molecular layer in the presence of glutamate receptor antagonists. Holding potential of 0 mV. The effect largely reversed after 20 min of washout. (C) cGABA (200 μM) did not significantly affect the EPSC evoked by stimulation of the perforant path. Holding potential of −80 mV.

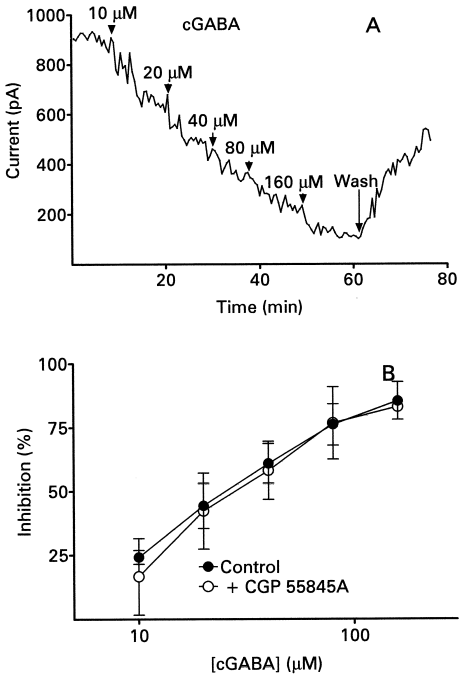

Fig. 2. cGABA depressed in a concentration-dependent manner the monosynaptic IPSC evoked by stimulation of the molecular layer. Stimuli were delvered every 30 s. (A) Increasing the concentration of cGABA progressively reduced the peak amplitude of the response recorded at a holding potential of 0 mV. (B) Cumulative concentration–response curve. The IC_{50} for cGABA was essentially the same in the absence (28 ± 9 μM, mean ± S.D., $n = 5$) and presence (32 ± 13 μM, mean ± S.D., $n = 4$) of the $GABA_B$ receptor antagonist CGP 55845A.

2.3. Muscimol-evoked currents

The recorded granule cell was visualized by including 100 μM fluorescein-dextran in the internal solution (excitation: 488 nm, 515 nm barrier filter). A glass micropipette (tip diameter ~5–10 μM) that contained 400 μM muscimol and 100 μM fluorescein-dextran dissolved in artificial cerebrospinal fluid was placed above the hippocampal slice over the inner third of the dentate molecular layer. Muscimol-containing solution was ejected from the micropipette with a Picospritzer (General Valve, Fairfield, NJ, USA; 6–10 psi), such that the solution contacted the surface of the slice just above the apical dendrite of the recorded cell. Muscimol was applied for ~300–500 ms every 2 min. The superfusion medium contained 5 μM NBQX and 50 μM D-AP5.

2.4. Recording of miniature inhibitory postsynaptic currents (mIPSCs)

In these experiments, the internal solution was modified: cesium gluconate was replaced with CsCl and QX-314 was omitted. The use of CsCl-filled electrodes shifted E_{Cl} to ~0 mV. The superfusion medium contained 5 μM NBQX, 50 μM D-AP5 and 1 μM tetrodotoxin. mIPSCs were recorded at a holding potential of −70 mV during a 150-s epoch. The liquid junction potential was considered

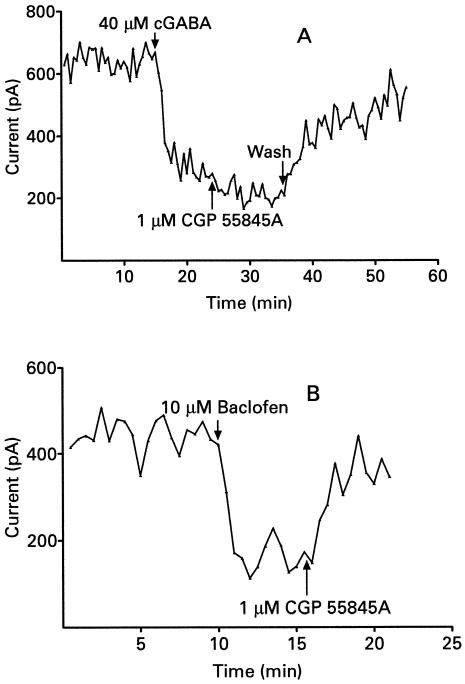

Fig. 3. CGP 55845A, a $GABA_B$ receptor antagonist, reversed depression of the monosynaptic IPSC by (±)-baclofen, but not by cGABA. Stimuli were delivered every 30 s. (A) CGP 55845A (1 μM) did not reverse the inhibitory effect of 40 μM cGABA. (B) The same concentration of CGP 55845A almost completely reversed the effect of 10 μM (±)-baclofen.

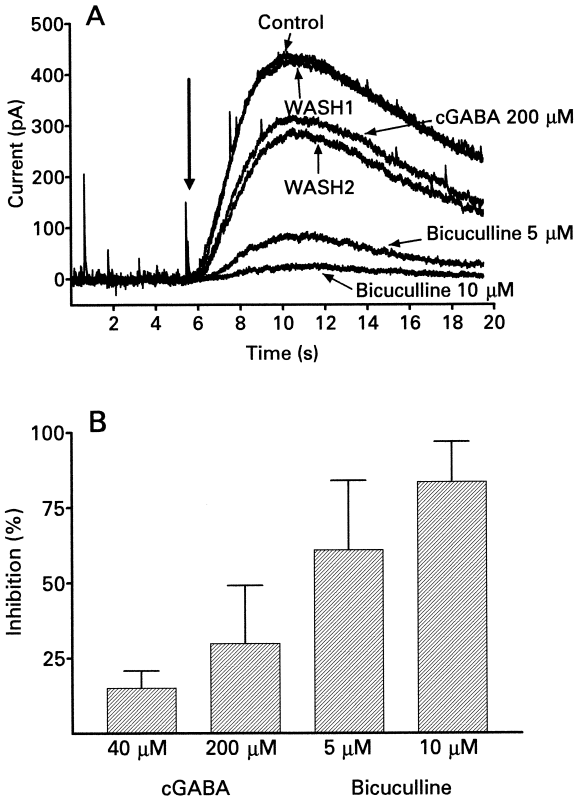

Fig. 5. cGABA (200 μM) modestly reduced $GABA_A$ receptor-mediated currents evoked by micropressure application of muscimol. (A) The granule cell was voltage-clamped at 0 mV and pulses of 400 μM muscimol were applied (arrow) for 300 ms every 2 min. Representative recordings illustrate a modest and reversible reduction in peak amplitude when 200 μM cGABA was added to the superfusion medium. After washout of cGABA (Wash1), bicuculline was applied at concentrations of 5 and 10 μM, 10 min apart. The effect of bicuculline largely reversed after washout of the antagonist (Wash2). (B) Results of seven to eight experiments with cGABA and 14–15 experiments with bicuculline demonstrate the much higher potency of bicuculline. Values are means ± S.D.

to be 0 mV with CsCl-based internal solution. Spontaneous events were recorded with pClamp6 and analyzed with functions incorporated in pClamp6 and Mini Analysis (Jaejin Software, Leonia, NJ, USA). The threshold for detection of a mIPSC was 6 pA.

2.5. Materials

L-Glutamate, γ-carboxy-2-nitrobenzyl ester (cGlu), cGABA and fluorescein-dextran (10,000 MW) were purchased from Molecular Probes (Eugene, OR, USA). D-Gluconic acid lactone, HEPES, EGTA, creatine phosphate, creatine phosphokinase and tetrodotoxin were obtained from Sigma (St. Louis, MO, USA). D-AP5 was purchased from Tocris Cookson (Bristol, UK) and bicuculline methiodide from Research Biochemicals (Natick, MA, USA). Cesium hydroxide (99.9%; 50 wt.%) and CsCl were pur-

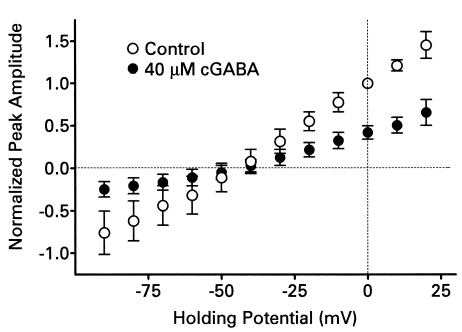

Fig. 4. Depression of the monosynaptic IPSC by cGABA was slightly greater at membrane potentials negative to the reversal potential. Current amplitudes were normalized to the amplitude of the synaptic response recorded at a holding potential of +10 mV. Values are means ± S.D. for five experiments.

Table 1
Effects of 40 μM cGABA on properties of mIPSCs recorded from dentate granule cells
See Fig. 6 for experimental details. Values are means ± S.D. for all mIPSCs recorded from each of the eight cells.

	Control	+cGABA	Difference (%)
Frequency (Hz)	1.4 ± 0.5	0.4 ± 0.2[a]	−71 ± 12
Amplitude (pA)	17.8 ± 9.1	9.6 ± 4.2[a]	−44 ± 9
10–90% Rise time (ms)	3.6 ± 0.8	4.6 ± 1.0	+28 ± 30
Decay time Constant (ms)	11.0 ± 2.2	8.1 ± 2.2[a]	−26 ± 12
Charge transfer (pC)	221 ± 137	104 ± 59[a]	−52 ± 5

[a] $P < 0.005$ (Student's *t*-test).

chased from Aldrich (Milwaukee, WI, USA). QX-314 chloride was obtained from Astra USA (Westborough, MA, USA) and Alomone Labs (Jerusalem, Israel). NBQX was a gift from Novo Nordisk (Måløv, Denmark). (±)-Baclofen and 3-*N*[1-(*S*)-(3,4-dichlorophenyl)ethyl]amino-2-(*S*)-hydroxypropyl-*P*-benzyl-phosphinic acid (CGP 55845A) were gifts from Ciba-Geigy (Basle, Switzerland).

3. Results

3.1. cGABA blocked inhibitory, but not excitatory, synaptic responses

Initial experiments utilized hippocampal slices from pilocarpine-treated rats. Addition of 200 μM cGABA to

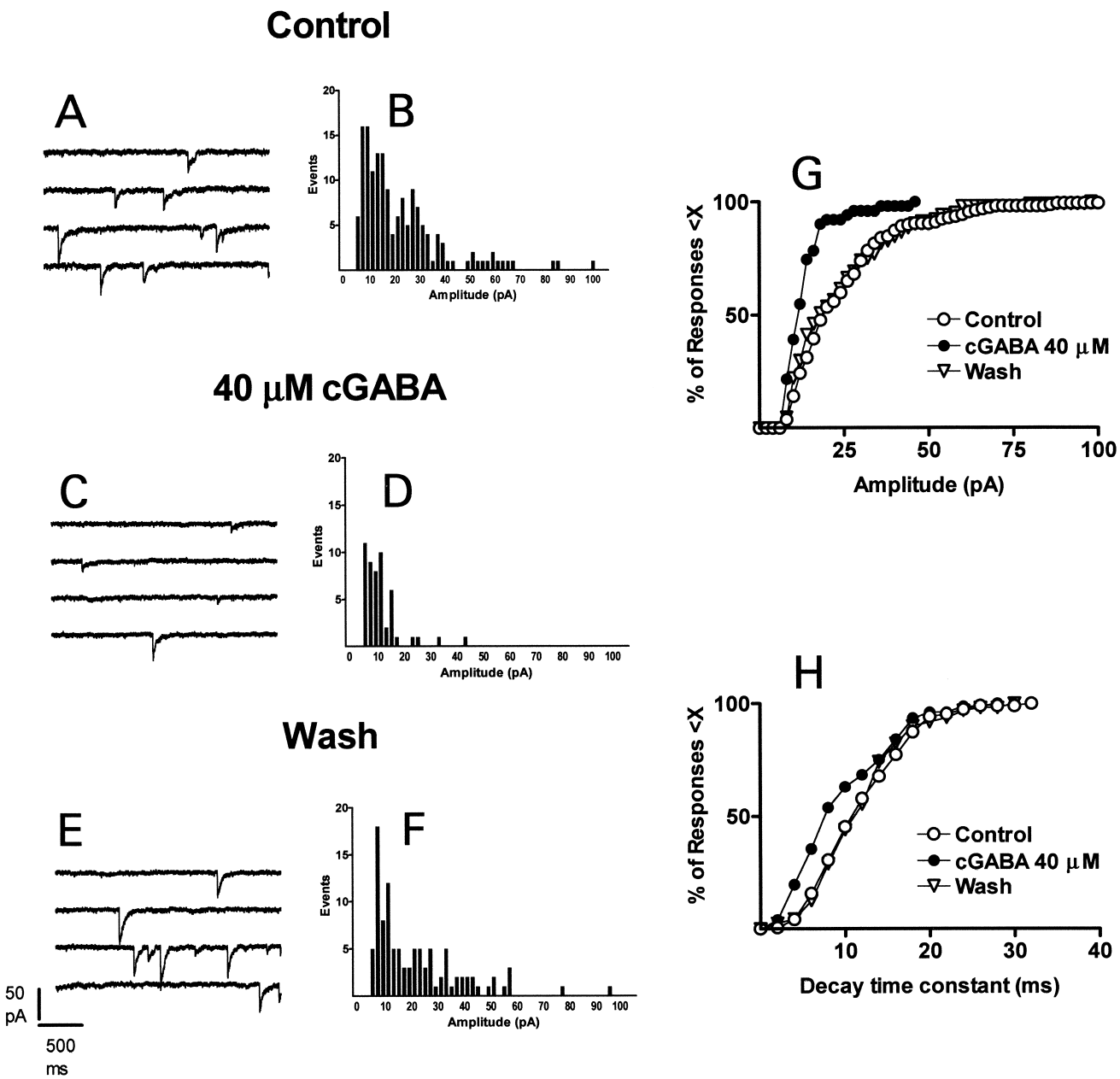

Fig. 6. cGABA (40 μM) reduced the frequency, peak amplitude and decay time constant of mIPSCs recorded from dentate granule cells. mIPSCs were recorded during a 150-s epoch at a holding potential of −70 mV. E_{Cl} was shifted to ~0 mV with use of a CsCl-based internal solution. Results shown are from a representative experiment. (A,C,E) Representative traces recorded during the control period, during a subsequent exposure to cGABA and after washout of cGABA. Transient inward currents are mIPSCs. (B,D,F) Amplitude histograms that illustrate both the smaller number of events recorded and the generally lower peak amplitude of the remaining events during superfusion with cGABA. (G) Cumulative response distribution of mIPSC amplitudes. Each value of peak amplitude is plotted against the percentage of IPSCs within that treatment group whose peak amplitude was smaller than that individual response (*X*). Leftward shift indicates smaller peak amplitudes in the presence of cGABA. (H) Cumulative response distribution of decay time constants. Leftward shift indicates generally shorter decay times in the presence of cGABA.

the superfusion medium nearly eliminated the polysynaptic feedback IPSC evoked by antidromic stimulation of the mossy fibers (Fig. 1A), whereas little did it affect the recurrent mossy fiber EPSC (not shown). The compound did not itself evoke a membrane current at holding potentials of either 0 or −80 mV. Based on these results, and because cGABA had been considered biologically inactive, we analyzed the action of cGABA in greater detail.

At a concentration of 160 μM, cGABA strongly depressed monosynaptic IPSCs evoked by stimulation of the dentate molecular layer (85 ± 7%, mean ± S.D., $n = 3$; Figs. 1B and 2). The onset of inhibition occurred within 5 min after the start of bath application, and the response returned to at least 80% of its original value within 30 min after washout of the compound (Figs. 1B and 3A). When cGABA was applied at cumulative concentrations 10 min apart (Fig. 2), it reduced the peak amplitude of the monosynaptic IPSC with an IC_{50} of 28 ± 9 μM (mean ± S.D., $n = 5$). In contrast, 200 μM cGABA did not affect EPSCs evoked by perforant path stimulation (3 ± 6% inhibition, mean ± S.D., $n = 4$; Fig. 1C). To determine whether the block of inhibitory synaptic transmission could be explained by activation of presynaptic $GABA_B$ receptors, the effect of cGABA was tested in the presence and absence of 1 μM CGP 55845A, a selective $GABA_B$ receptor antagonist (Davies et al., 1993; Jarolimek et al., 1993). CGP 55845A did not reverse the inhibitory effect of cGABA on GABA transmission (Fig. 3), nor did it change its IC_{50} (Fig. 2; $n = 4$). In the same series of experiments, however, CGP 55845A largely reversed the inhibitory effect of 10 μM (±)-baclofen (Fig 3; 58 ± 16% inhibition in the absence and 13 ± 8% inhibition in the presence of 1 μM CGP 55845A, means ± S.D., $n = 6$).

To determine whether reduction of the monosynaptic IPSC by cGABA was voltage-dependent, the holding potential was changed in 10-mV steps from −90 to +30 mV (Fig. 4). cGABA (40 μM) depressed the response to a slightly greater degree at membrane potentials negative to the reversal potential (67 ± 8% at −80 mV and 58 ± 7% at +10 mV; means ± S.D., $n = 5$). It did not affect E_{Cl}.

3.2. cGABA modestly reduced the response to applied muscimol

To evaluate the possibility that cGABA antagonizes the activation of $GABA_A$ receptors, we studied its effect on responses to muscimol, a selective $GABA_A$ receptor agonist. The kinetics of the muscimol-evoked outward current were slow (Fig 5), due presumably to the slow penetration of muscimol through the slice. Micropressure application of muscimol every 2 min evoked currents of essentially constant amplitude. cGABA reduced the amplitude of muscimol-evoked currents in a concentration-dependent manner (Fig. 5). Its inhibitory effect was rather modest, however. Reductions of 15 ± 6% and 30 ± 19% (means ± S.D., $n = 7–8$) were obtained with concentrations of 40 and 200 μM, respectively. In the same experiments, the $GABA_A$ receptor antagonist bicuculline reduced muscimol-evoked currents by 61 ± 23% at a concentration of 5 μM and 84 ± 13% at a concentration of 10 μM (means ± S.D., $n = 14–15$) (Fig. 5).

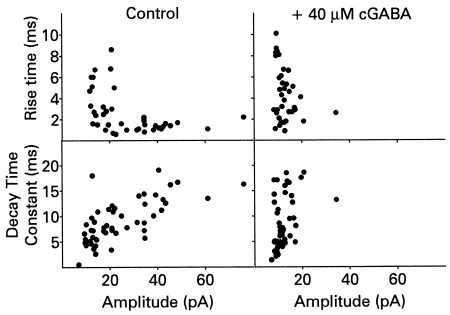

Fig. 7. Rise times (10–90%) and decay time constants of individual mIPSCs plotted as a function of peak amplitude. Results shown are from a representative experiment. mIPSCs with high amplitude and short rise times dropped out in the presence of cGABA.

3.3. cGABA had complex effects on mIPSCS

In an attempt to differentiate presynaptic from postsynaptic actions of cGABA, we studied mIPSCs in eight dentate granule cells. mIPSCs occurred at a frequency of 1.4 Hz in the absence of cGABA, and their mean peak amplitude was 17.8 pA. cGABA (40 μM) reduced the mean frequency of mIPSCs by 71%, their mean peak amplitude by 44%, their mean decay time constant by 26% and charge transfer per event by 52% (Table 1; Fig. 6). Mean rise time increased by 28%, but this change was not statistically significant ($0.1 > P > 0.05$, Student's t-test). Plots of rise time and decay time constant against response amplitude revealed that cGABA reduced the number of mIPSCs with both peak amplitudes > 20 pA and 10–90% rise times < 2 ms (Fig. 7).

3.4. cGlu little affected inhibitory synaptic transmission

cGABA is marketed as the trifluoroacetate salt. To test the possible effect of trifluoroacetate, as well as of the caging group, on the monosynaptically evoked IPSC, we utilized cGlu, another carboxy-2-nitrobenzyl ester marketed as the trifluoroacetate salt. cGlu (200 μM) did not significantly reduce the peak amplitude of the monosynaptic IPSC (15 ± 9%, mean ± S.D., $n = 5$).

4. Discussion

cGABA was reported to lack $GABA_A$ receptor agonist or antagonist action when tested on mouse cortical cultures at concentrations as high as 500 μM (Gee et al., 1994). We confirm its lack of agonist activity, but cGABA moderately depressed the response of dentate granule cells to

applied muscimol. This result indicates that cGABA can act as a $GABA_A$ receptor antagonist, at least in our system. To our knowledge, the effects of cGABA on synaptic transmission have not been studied previously. In contrast to its moderate depression of responses to muscimol, cGABA strongly and potently blocked inhibitory transmission in the rat dentate gyrus without affecting excitatory transmission. cGABA depressed both monosynaptically and polysynaptically evoked GABA inhibition, and it also reduced the frequency, amplitude and decay time constant of mIPSCs. Its actions appeared not to result from exposure of the tissue to the trifluoroacetate counterion, because the trifluoroacetate salt of cGlu did not exhibit comparable effects. For the same reason, the actions of cGABA did not depend entirely on the carboxy-2-nitrobenzyl caging group. Evidently, both the GABA and the carboxy-2-nitrobenzyl portions of the molecule are required for depression of inhibitory synaptic responses.

Further studies are required to explain the differing effects of cGABA on $GABA_A$ receptor-mediated responses of dentate granule cells in the hippocampal slice and cortical neurons in dissociated culture. Because the effect of cGABA on muscimol-evoked currents in dentate granule cells required rather high concentrations to demonstrate clearly (30% depression at 200 μM), the compound may simply be a less potent antagonist in cultured cortical neurons. At least 16 genes encode subunits of the $GABA_A$ receptor, allowing the assembly of many receptor subtypes with differing kinetics, cell-specific expression and pharmacology (Möhler et al., 1995). One possibility is that mature dentate granule cells and cultured cortical neurons express $GABA_A$ receptors composed of different subunits with different affinities for cGABA.

The depressant action of cGABA on inhibitory synaptic transmission closely resembles that of phenothiazine-type antipsychotic drugs, such as chlorpromazine. Chlorpromazine was reported to decrease the binding rate and increase the unbinding rate of GABA to $GABA_A$ receptors in rat hippocampal cultures (Morzymas et al., 1999). The drug much more strongly blocked synaptic responses and responses to rapid (milliseconds) application of GABA than responses to slower (seconds) applications of GABA. Similarly, cGABA more potently blocked spontaneous and evoked IPSCs than currents evoked by application of muscimol for hundreds of milliseconds. Furthermore, cGABA, like chlorpromazine, reduced the amplitude, mean charge transfer and decay time constant of mIPSCs without significantly changing rise time. Finally, phenothiazines block $GABA_A$ receptors slightly more effectively at negative membrane potentials and do not alter E_{Cl} (Zorumski and Yang, 1988). Whether cGABA produces these effects in the same manner as chlorpromazine, by altering $GABA_A$ receptor kinetics, remains to be determined. A chlorpromazine-like action of cGABA could also explain, in part, its marked reduction of mIPSC frequency, because a reduction in peak amplitude would likely cause some of the smaller mIPSCs to become lost in the background noise. Due to the magnitude of the reduction in mIPSC frequency, however, we cannot exclude the possibility that cGABA also acts in some way to reduce GABA release. If cGABA does reduce GABA release, our data suggest it must do so through a mechanism other than activation of $GABA_B$ receptors.

Alternatively, or in addition, cGABA may block certain GABA pathways while little affecting others. The data presented in Fig. 7 are consistent with a selective loss of high amplitude mIPSCs with short rise times. Dentate granule cells receive inhibitory innervation from at least six distinct populations of GABA interneurons (Freund and Buzsáki, 1996). Each GABA projection contacts a unique portion of the cell, either the soma, axon initial segment or a specific dendritic domain. Tonic inhibition of granule cells originates mainly from GABA projections that terminate on or close to the soma (Soltesz et al., 1995). The larger mIPSCs recorded with a somatic electrode are also likely to originate mainly on or near the soma, because they would be the least attenuated by filtering through the membrane time constant (Spruston et al., 1994). Results of Soltesz et al. (1995) suggest that mIPSCs > 20 pA with 10–90% rise times < 2 ms arise within ~ 50 μm from the granule cell body layer. Thus, cGABA may preferentially block somatic inhibitory synapses, including those made by basket cells. Paired whole cell or intracellular recordings are needed to evaluate this possibility.

cGABA is used as a tool to evoke $GABA_A$ receptor-mediated responses with high spatial and temporal resolution (Gee et al., 1994; Dittman and Regehr, 1997). For example, effects of GABA at different locations in the dendritic tree can be studied by exposing each location to a brief pulse of highly focused UV light in the presence of cGABA. In experiments of this type, the effects of cGABA discovered in the present study would not be readily detectable, because the current evoked by uncaged GABA can still be observed. To the extent that cGABA antagonizes the action of GABA upon $GABA_A$ receptors, the evoked current would simply be less than might otherwise be expected. However, it is critical to take the effects of cGABA into account when recording synaptic responses during such experiments. In addition to its use as a source of GABA, cGABA itself may be a useful tool for research on GABA mechanisms, because it exhibits a complex of actions on inhibitory synaptic transmission that are not shared by other structural analogues of GABA.

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