Properties of Unitary IPSCs in Hippocampal Pyramidal Cells Originating From Different Types of Interneurons in Young Rats

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INTRODUCTION

Granule cells of the dentate gyrus and pyramidal cells of Ammon’s horn make up a well-defined excitatory circuit in the hippocampus (Lorente de No 1934; Ramon y Cajal 1911). In addition, interneurons form complex local networks within the hippocampus (Amaral 1978; Buhl et al. 1994a; Lacaille et al. 1989; Lorente de No 1934; Ramon y Cajal 1911; Sik et al. 1995). Most interneurons are inhibitory, using γ-aminobutyric acid (GABA) as neurotransmitter (Ribak et al. 1978; Woodson et al. 1989), and they critically offset the excitatory activity in principal cells (Andersen et al. 1964a,b; Kandel et al. 1961; Traub et al. 1987).

Postsynaptic GABA mechanisms have been extensively characterized in hippocampal pyramidal cells (Nicoll et al. 1990; Sivilotti and Nistri 1990). Synaptically released GABA opens postsynaptic GABA A receptors/channels permeable to Cl⁻, to produce a rapid postsynaptic response (Alger and Nicoll 1982a,b). Postsynaptic GABA A mechanisms may be heterogeneous, because selective activation of subsets of inhibitory afferents with the use of minimal stimulation elicits inhibitory postsynaptic currents with different physiological and pharmacological properties (Lambert and Wilson 1993; Pearce 1993). GABA also binds to postsynaptic GABA B receptors, leading to G protein activation and K⁺ channel opening, to generate a slow postsynaptic response (Dutar and Nicoll 1988a,b; Newberry and Nicoll 1985). Multiple types of inhibitory interneurons have been identified in the CA1 region on the basis of their morphology, membrane properties, and local circuit interactions (Buhl et al. 1994a; Kawaguchi and Hama 1987; Lacaille and Schwartzkroin 1988a,b; Lacaille et al. 1987; McBain et al. 1994; Schwartzkroin and Mathers 1978). Furthermore, these interneuron subtypes are segregated in distinct layers in the CA1 region. The precise role of these various subtypes of interneurons in generating GABA inhibition of pyramidal cells remains largely unknown. Direct stimulation of individual interneurons in or near the pyramidal cell layer elicits GABA A inhibitory postsynaptic potentials (IPSPs) in pyramidal cells (Buhl et al. 1994a; Miles 1990). The synaptic mechanisms involved in the inhibition of pyramidal cells by other subtypes of interneurons remains to be demonstrated, although some evidence suggests that specific subpopulations of interneurons may be responsible for GABA A inhibition (Lacaille and Schwartzkroin 1988b; Lacaille et al. 1989; Samulack and Lacaille 1993; Segal 1990; Williams and Lacaille 1992).

The present study was designed to examine the physiological and pharmacological properties of unitary synaptic currents in pyramidal cells originating from different interneurons. Cells were visually identified in hippocampal slices and loose cell-attached stimulation of individual interneurons was performed during whole cell recordings from pyra-
midal cells (Edwards et al. 1990). Subtypes of interneurons, located near the pyramidal cell layer (stratum pyramidale, PYR), in distal apical (stratum lacunosum-molecular/radiatum border, LM) and basal (stratum oriens, OR) dendritic layers, were selectively activated. These different subtypes of hippocampal interneurons were found to produce unitary inhibitory postsynaptic currents (uIPSCs) in CA1 pyramidal cells via GABAA receptors and Cl\textsuperscript{−} channels, but with heterogeneous properties. In addition, uIPSCs appeared under tonic presynaptic inhibition via heterosynaptic GABAB receptors.

METHODS

Slices

Hippocampal slices (300 \( \mu \)m thick) were prepared from young (14–21 days) male Sprague-Dawley rats as previously described (Ouardouz and Lacaille 1995; Williams et al. 1994). Slices were maintained at room temperature (22–24°C) in a container filled with artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH2PO4, 2 MgSO4, 2 CaCl2, 26 NaHCO3, and 10 \( \alpha \)-glucose saturated with 95% O2-5% CO2. After an initial incubation period of 1 h, a slice was placed in a recording chamber and maintained submerged during continuous perfusion at 3–4 ml/min with oxygenated ACSF (Edwards et al. 1989). The recording chamber was mounted on an upright microscope (Zeiss Axioskop) equipped with a long-range water-immersion objective (x40), Nomarski optics, and a near infrared charge-coupled device camera ( Cohu 6500) to allow visual identification of various subtypes of interneurons and pyramidal cells in the CA1 area.

Recoding and stimulation

Whole cell recordings were made from CA1 pyramidal cells with the use of patch electrodes (5–10 M\( \Omega \)) filled with either of two solutions containing (in mM) 140 potassium gluconate, 5 NaCl, 2 MgCl2, 10 \( N,N,N,N\)-tetraethylammonium-\( N\)-2-ethanesulfonic acid (HEPES), 0.5 ethylene glyco-bis(\( \beta \)-aminoethyl ether)-\( N,N,N,N\)-tetraacetic acid (EGTA), 2 adenosine \( 5'\)-triphosphate (ATP)-Tris, and 0.4 guanosine \( 5'\)-triphosphate (GTP)-Tris, pH adjusted to 7.3 with KOH; or 2) 140 cesium methanesulfonate, 5 NaCl, 1 MgCl2, 10 HEPES, 1 EGTA, 2 ATP-Tris and 0.4 GTP-Tris, pH adjusted to 7.3 with CsOH. Voltage-clamp recordings were made with the use of an Axopatch 1D amplifier (Axon Instruments) with low-pass filtering at 10 kHz \((-3\,dB)\). Series resistance was compensated to 80% and regularly monitored for constancy throughout the experiment. The mean series resistance was 18.1 ± 1.1 (SE) M\( \Omega \) \((n = 64)\). Recordings were digitized at 22 kHz for storage on a video cassette recorder (Neurocorder DR-886), and also analyzed with a PC-DOS microcomputer-based data acquisition system (TL-125 and pClamp, Axon Instruments). For stimulation of individual interneurons, a patch pipette \((5–10\,M\Omega)\) filled with ACSF was first positioned on the soma of a visually identified interneuron and gentle suction was applied (Edwards et al. 1990). In this loose cell-attached configuration, constant current stimulation \((1\,ms, 0–8\,\mu A, 0.2\,Hz)\) was applied with a stimulus isolation unit (WPI), in the presence of the glutamate receptor antagonists 20 \( \mu \)M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, RBI) and 50 \( \mu \)M \((\pm)\)-2-amino-5-phosphopentoic acid (AP-5, RBI). In some control experiments, biphasic monosynaptic IPSPs were recorded from pyramidal cells in slices taken from mature rats \((175\,g)\) with the use of potassium gluconate containing electrodes. Monopolar electrical stimulations \((0–200\,\mu A, 0.05\,ms)\) were delivered with a tungsten microelectrode positioned in dendritic layers and synaptic responses were monitored in current clamp.

For the analysis of the properties of uIPSCs, the peak amplitude, onset latency, 10–90% rise time, and decay time constant were measured on averaged responses \((n = 16\,traces)\). Individual traces with superimposed spontaneous IPSCs or with failures were rejected from analysis. The peak amplitude was taken as the difference in mean current between a 1-ms time window before the stimulus artifact and an 0.5-ms window at the peak of the averaged IPSC. The decay time constant of uIPSCs was determined by exponential fitting \((pClamp)\). In all cases, visual inspection indicated that the decay of averaged uIPSCs was well fitted by a single exponential. Group measures were expressed as means ± SE. Statistical differences between means of groups was determined with Student’s \( t \)-tests \((significance\ level P < 0.05)\). For amplitude distributions of uIPSCs, statistical differences were assessed with Kolmogorov-Smirnov tests. The GABA antagonists bicuculline methiodide (Sigma, 25 \( \mu \)M) and CGP 55845A (Ciba-Geigy, 5 \( \mu \)M) were bath applied in ACSF.

RESULTS

uIPSCs

CA1 pyramidal cells and interneurons were visually distinguished in rat hippocampal slices by the location and morphology of their somata, and their dendritic arborizations (Morin et al. 1996; Williams et al. 1994). A patch pipette containing extracellular solution was positioned in the loose-cell-attached configuration onto an interneuron for stimulation, and whole cell voltage-clamp recordings were obtained from a CA1 pyramidal cell in the presence of the glutamate receptor antagonists 20 \( \mu \)M CNQX and 50 \( \mu \)M AP-5 to eliminate excitatory synaptic activity \((Fig. 1; \text{see methods})\). With the pyramidal cell held at +20 mV, the stimulus intensity was increased until an IPSC was evoked in an all-or-none fashion \((Fig. 1, B \text{and C})\). Further increases in stimulation intensity did not result in increases in IPSC amplitude, suggesting that a single presynaptic cell had been recruited. These IPSCs were referred to as uIPSCs. uIPSCs evoked by all interneurons fluctuated in amplitude, and occasionally showed failure of transmission. In some cases, particularly for LM interneurons, uIPSCs disappeared abruptly after a few minutes as a result of cell damage; these recordings were not included in the study. With the use of this approach, 64 synaptic connections were identified between pyramidal cells and interneurons in PYR \((n = 22)\), OR \((n = 18)\), and LM \((n = 24)\).

Properties of uIPSCs

uIPSCs evoked in pyramidal cells differed depending on the type of interneuron stimulated. In initial recordings with intracellular solution containing potassium gluconate, uIPSCs evoked by stimulation of OR \((n = 4)\) and PYR \((n = 2)\) interneurons were generally similar, but those elicited by activation of LM interneurons \((n = 5)\) were smaller in amplitude and had slower time courses \((data\ not\ shown)\). These properties were then characterized in cells held at +20 mV with the use of cesium methanesulfonate intracellular solution to block K\textsuperscript{+} conductances and improve space clamp \((Fig. 2)\). The mean peak amplitude was not significantly different for averaged uIPSCs evoked by stimulation of OR \((78.9 ± 19.1\,pA;\ range: 14.6–208.9\,pA;\ n = 11)\) and PYR \((112.3 ± 18.6\,pA;\ range: 49.0–288.0\,pA;\ n = 18)\) interneurons \((Fig. 2; P > 0.05)\). However, the peak amplitude of uIPSCs evoked from LM interneurons \((33.2 ± 8.4\,pA;\)
stimulation of OR and PYR interneurons showed a similar performed to verify that postsynaptic GABA B responses between responses from PYR and LM interneurons (P not significant different (−58.5 ± 2.6 mV, range: −45.3 to −70.4 mV, n = 13, P > 0.05) (Fig. 3). With this recording solution, the mean Erev of uIPSCs for each cell type were −58.3 ± 12.5 mV for PYR (n = 9), −57.5 ± 12.5 mV for OR (n = 2), and −62.5 ± 2.5 mV for LM (n = 2) cells. These Erev values were generally more depolarized than the calculated chloride equilibrium potential in our experimental conditions (−68 mV for potassium gluconate solution and −74 mV for cesium methanesulfonate solution). However, the lack of significant difference between the mean Erev with the use of these two recording solutions suggested that potassium conductances were not involved in these uIPSCs. The current-voltage relation of uIPSCs was nonlinear (Fig. 3) and showed outward rectification in 14 of 22 cell tested. The kinetics of uIPSCs appeared voltage sensitive (Fig. 3). The mean rise time of uIPSCs for all cell types was not significantly different at −20 mV (4.5 ± 0.9 ms) and +40 mV (3.8 ± 0.8 ms, n = 10; P > 0.05). The mean decay time constant was significantly slower at +40 mV (53.9 ± 7.2 ms) than −20 mV (39.8 ± 7.2 ms, n = 13, P < 0.05).

GABA_A uIPSCs

uIPSCs evoked by different interneuron subtypes were all mediated by GABA_A receptors. During bath application of 25 μM bicuculline, the mean peak amplitude of uIPSCs, recorded with potassium gluconate intracellular solutions, was blocked up to 3.5 ± 4.4% of control (n = 7, P < 0.05). uIPSCs were antagonized for all presynaptic cell types tested (n = 2 PYR, 2 OA and 3 LM cells). Similarly, with the use of cesium methanesulfonate intracellular solutions, bicuculline reversibly blocked uIPSCs evoked from all cell types (Fig. 4; mean amplitude in bicuculline 2.9 ± 3.1% of control, n = 6 PYR, 4 OA, and 3 LM cells; P < 0.05).

Because bicuculline completely blocked uIPSCs evoked by different interneurons, positive control experiments were performed to verify that postsynaptic GABA_A responses could be recorded in our experimental conditions. Compound monosynaptic IPSPs were recorded in current-clamp experiments from CA1 pyramidal cells in slices from mature rats (175 g) with the use of potassium gluconate recording solution (Fig. 5). In these conditions, typical biphasic GABA_A and GABA_B IPSPs were evoked, which consisted of a fast component reversing at −73.5 ± 6.5 mV and a slow component with a null potential of −82.5 ± 5.0 mV (n = 2, Fig. 5A). In the presence of bicuculline (25 μM), only slow monosynaptic IPSPs (Fig. 5B) were present, showing a reversal potential of −79.3 ± 3.7 mV (n = 3).

Presynaptic GABA_A inhibition

uIPSCs evoked by stimulation of LM cells, and recorded with potassium gluconate pipette solutions, were larger in amplitude (129.5 ± 3.4% of control, n = 2) during bath application of the GABA_A receptor antagonist CGP 55845A.
FIG. 2. Properties of uIPSCs evoked by stimulation of different interneuron subtypes. A–C: representative examples of uIPSCs ($V_{\text{hold}} = 20 \text{ mV}$) evoked by stimulation (0.2 Hz) of interneurons near stratum pyramidale (PYR, A), in OR (B), and near the stratum radiatum and lacunosum-moleculare border (LM, C). Superimposed single traces (left) and averaged uIPSCs (right) were similar for responses evoked by activation of PYR (A) and OR (B) cells, but were smaller in amplitude and had a slower time course in responses elicited by stimulation of LM cells (C). D–F: summary histograms, for all cells tested, of mean $\pm$ SE peak amplitude (D), rise time (E), and decay time constant (F) of averaged uIPSCs evoked from different interneuron subtypes. Asterisks: significant differences in amplitude, rise time, and decay of uIPSCs between cell types. G: graph of decay time constant as a function of rise time for uIPSCs of all cell types. The positive correlation between decay and rise time was not statistically significant.

The effects of CGP 55845A were further characterized with the use of cesium methanesulfonate-filled electrodes. The mean peak amplitude of averaged uIPSCs was significantly and reversibly increased to 141.6 $\pm$ 12.8% of control ($n = 10$, $P < 0.05$) in CGP 55845A (Fig. 6). More individual uIPSCs of large amplitude were seen in CGP 55845A than in control ACSF (Fig. 7). For individual cell types, the mean amplitude of uIPSCs was significantly increased in CGP 55845A for three of four PYR, three of four OR, and two of two LM interneurons tested. The effect of the GABA$_B$ receptor antagonist on the amplitude distribution of uIPSCs was examined (Fig. 7). The cumulative probability distribution of the peak amplitude of uIPSCs was significantly shifted toward larger values in the presence of CGP 55845A compared with control (Fig. 7, Kolmogorov-Smirnov test, $P < 0.05$). Such a significant shift was seen in 6 of 10 presynaptic cells tested ($n = 2$ of 4 PYR, 3 of 4 OR, and 1 of 2 LM interneurons). In the presence of CGP 55845A, the amplitudes of the larger uIPSCs were greater than in control (Fig. 7B).

**Paired pulse stimulation**

Because monosynaptic IPSCs in CA1 pyramidal cells show paired pulse GABA$_B$ depression (Davies et al. 1990), we examined whether uIPSCs displayed paired pulse inhibition at interstimulus intervals of 100 and 200 ms during recordings with cesium methanesulfonate intracellular solution. No significant differences were found between the amplitude of first and second averaged uIPSCs or between ensembles of first and second uIPSCs (Fig. 8A) for all presynaptic cells tested ($n = 5$ PYR and 4 OR cells). The difference between the mean amplitude of averaged uIPSC$_1$ (127.2 $\pm$ 24.9 pA) and uIPSC$_2$ (135.0 $\pm$ 26.9 pA) was not significant.
UNITARY IPSCs IN CA1 PYRAMIDAL CELLS

FIG. 3. Current-voltage relation of uIPSCs. A: superimposed averaged uIPSCs evoked by stimulation of a PYR cell at holding potentials between -80 and +30 mV, recorded with cesium methanesulfonate solution. B: plot of peak amplitude vs. holding potential of averaged uIPSCs shown in A. The reversal potential was close to the Cl⁻ equilibrium potential. C and D: graphs of mean decay time constant (C) and rise time (D) vs. membrane potential for pooled data (n = 13 and 10 cells, respectively). Decay time constant was significantly increased with depolarization (C; asterisk indicates significant statistical difference, P < 0.05), but rise time did not significantly change (D).

(P > 0.05). However, because at excitatory synapses, the amplitudes of the second responses evoked by paired pulse stimulation are dependent on the probability of transmitter release during the first responses, the paired pulse ratio of individual responses (uIPSC₂/uIPSC₁ × 100) was examined as a function of the amplitude of uIPSC₁ (Debanne et al. 1996). In all cells tested, a significant negative correlation was found between paired pulse ratio and uIPSC₁ amplitude, which was well fitted by a hyperbolic function [y = (a/x) + b] (Debanne et al. 1996) (Fig. 8, C and D). When the quantal content of the first response was low (i.e., amplitude of uIPSC₁ < mean amplitude of all uIPSC₁), the amplitude of uIPSC₂ was larger than that of uIPSC₁ (Fig. 8, B and C). For the nine cells examined, when uIPSC₁ was smaller than the mean of all uIPSC₁, the mean paired pulse ratio was 154.0 ± 8.0%. In addition, the mean amplitude of uIPSC₂ for this group of responses (167.05 ± 25.1 pA) was significantly larger than the mean amplitude of all uIPSC₁ (138.7 ± 22.4 pA). Thus analysis of individual responses revealed paired pulse facilitation when the quantal content of the first response was low. There was a tendency toward paired pulse inhibition when the quantal content of the first response was high, but this was not significant (Fig. 8D). For all cells, the mean paired pulse ratio was 90.8 ± 4.0% when the amplitude of uIPSC₁ was larger than the mean amplitude of all uIPSC₁. But, for this group of responses, the mean amplitude of uIPSC₂ (142.8 ± 24.4 pA) was not significantly different from the mean amplitude of all uIPSC₁, indicating an absence of paired pulse inhibition in these conditions.

DISCUSSION

The principal findings of the present study are that activation of individual interneurons in PYR, OR, and LM layers of the CA1 region evoked uIPSCs in pyramidal cells that were blocked by the GABA_A antagonist bicuculline, were potentiated by the GABA_B antagonist CGP 55845A, showed a reversal potential near -55 mV, and were unaltered by the K⁺ conductance blocker cesium. Thus all unitary responses were mediated via GABA_A receptors and Cl⁻ channels. uIPSCs originating from LM interneurons were smaller in amplitude and had a slower time course. Therefore synaptic mechanisms originating from different interneurons may be
berry and Nicoll 1984), some feed-forward inhibitory pathways to pyramidal cells may involve solely GABA<sub>A</sub> postsynaptic mechanisms. Because stellate cells also project to dentate gyrus and CA3 area (Kunkel et al. 1988; Williams et al. 1994), the synchronization they provide across hippocampal regions may also involve GABA<sub>A</sub> mechanisms.

No postsynaptic GABA<sub>B</sub> components were detected in uIPSCs, in contrast to previous suggestions of GABA<sub>B</sub> inhibition by LM interneurons (Williams and Lacaille 1992). However, many factors may account for these differences. First, some LM cells did not tolerate the present stimulation procedure. Thus different presynaptic cells may have been sampled. Second, animals of different ages were used (juvenile vs. mature). Regional differences have been observed in the development of postsynaptic GABA<sub>B</sub> responses. Postsynaptic GABA<sub>B</sub> receptors became functional ~11 days postnatally in rat somatosensory and visual cortex (Luhmann and Prince 1991), and ~6 days postnatally in rat CA3 pyramidal cells (Gaiarsa et al. 1995), but only between 12–30 days postnatally in rabbit CA1 pyramidal cells (Janigro and Schwartzkroin 1988). Thus, in the present study, the absence of postsynaptic GABA<sub>B</sub> components could be related to their late maturation in CA1 pyramidal cells. Third, different methods of stimulation, single-cell versus local glutamate, were used. Indeed, strong electrical stimulation evokes GABA<sub>B</sub> responses, whereas weak stimulation, or spontaneous activation of single cells or synapses, elicits GABA<sub>A</sub> responses (Dutar and Nicoll 1988a,b; Otis and Mody 1992a). Also, inhibition of GABA uptake can recruit GABA<sub>B</sub> components in evoked IPSPs, but not in miniature events (Thompson and Gähwiler 1992). Thus GABA<sub>B</sub> receptor activation may require a large release of GABA and a cofactor (Mody et al. 1994). Specialized interneurons responsible for GABA<sub>B</sub> inhibition have, however, been reported (Segal 1990; Sugita et al. 1992).

**Properties of uIPSCs**

The similar properties of uIPSCs originating from PYR and OR interneurons suggest homogeneous synaptic mechanisms for these cells despite large variations in their postsynaptic target sites on pyramidal cells. PYR interneurons contact pyramidal cell somata and proximal dendrites (basket cells, Buhl et al. 1994a; Sik et al. 1995), axon initial segments (axoaxonic cells, Buhl et al. 1994a,b), and basal and apical dendrites (bistratified cells, Buhl et al. 1994a). In contrast, OR interneurons contact basal and apical dendrites of pyramidal cells (vertical cells, Lacaille et al. 1987; Sik et al. 1995), or specifically their distal apical dendrites (horizontal cells, McBain et al. 1994). The similar kinetics of unitary synaptic currents from PYR and OR interneurons observed in the present study are in contrast to the fast and slow time course of synaptic potentials generated by interneurons making somatic and dendritic synapses that were reported in current-clamp recordings (Buhl et al. 1994a). This would suggest that the different time course of synaptic potentials originating from dendritic and somatic synapses (Buhl et al. 1994a) may be due to electrotonic filtering of more distant dendritic synapses and not to differences in underlying synaptic currents. Finally, in the present study, the observed reversal potentials of uIPSCs (about ~55
FIG. 5. Compound monosynaptic inhibitory postsynaptic potentials (IPSPs) in CA1 pyramidal cells of mature rats. A: in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and (±)2-amino-5-phosphonopentanoic acid (AP-5), electrical stimulation of inhibitory fibers elicited biphasic IPSPs consisting of an early (●) and a late (■) component. The early IPSP reversed between −52 and −86 mV, whereas the late IPSP became null near −86 mV. Each trace is an average of 3 responses.

Properties of uIPSCs originating from LM interneurons

The reduced amplitude and slower kinetics of uIPSCs originating from LM cells suggest that postsynaptic mechanisms may be different between interneurons. Several reasons could explain these uIPSC differences. First, they could be due to dendritic filtering in poorly clamped dendrites (Spruston et al. 1993), because LM cell axons contact mostly pyramidal cell apical dendrites (stellate cells, Kunkel et al. 1988; Lacaille and Schwartzkroin 1988a; Misgeld and Frotscher 1986). However, the K⁺ conductance blocker cesium was used to improve voltage control. Also, the similar reversal potential of uIPSCs and the nonsignificant correlation between rise time and decay are suggestive of appropriate dendritic voltage control (Hestrin et al. 1990; but see Spruston et al. 1993). More importantly, because the electrotonic location of some OR interneuron synapses may be equally or more distant from pyramidal cell somata than LM cell synapses (Lacaille et al. 1987; McBain et al. 1994; Sik et al. 1995), and because uIPSCs from OR interneurons displayed faster kinetics and larger amplitude than their LM interneuron counterparts, poor voltage-clamp control cannot explain totally the differences in kinetics and properties. Identification of synaptic contact sites by individual interneurons combined with uIPSC characterization should help resolve this issue.

Differences in postsynaptic receptor mechanisms could account for uIPSC differences. Two types of GABA_A IPSCs have been distinguished in pyramidal cells by stimulation of different layers: a fast furosemide-sensitive and a slow furosemide-insensitive IPSC (Pearce 1993). Additionally, skewed distributions of miniature IPSC decay rate, suggestive of distinct underlying populations, have been observed in hippocampal granule cells (Otis and Mody 1992b). Our results are consistent with two such distinct GABA_A conductions, but also suggest that PYR and OR interneurons may be involved in fast GABA_A responses and LM cells in slow
responses. Single-channel recordings and rapid applications of GABA in hippocampal and cerebellar granule cells have uncovered channels with fast and slow kinetics, compatible with biexponentially decaying GABA$_A$ IPSCs (Edwards et al. 1990; Puia et al. 1994). However, uIPSC decay was monoexponential and lacking a fast component in the present study. If the slower kinetics of uIPSCs of LM interneurons reflect different receptor mechanisms, then these slower receptor mechanisms could contribute, in addition to electrotropic factors affecting distant dendritic synapses, to the slow time course and attenuation of synaptic potentials generated by LM interneurons (Lacaille and Schwartzkroin 1988b).

Heterogeneous postsynaptic currents originating from different interneurons may suggest some functional specialization. Synaptic currents with rapid kinetics, originating from PYR and OR interneurons, may preferentially curtail rapid non-N-methyl-D-aspartate (NMDA)-mediated excitatory events (Hestrin et al. 1990). Synaptic currents with slower kinetics, originating from LM interneurons, may effectively inhibit slower NMDA-mediated excitatory events (Davies et al. 1991; Hestrin et al. 1990) or local Ca$^{2+}$ responses (Masukawa and Prince 1984; Miyakawa et al. 1992).

**Presynaptic inhibition of uIPSCs**

The potentiation of uIPSCs by the GABA$_B$ antagonist CGP 55845A (Davies et al. 1993) in the presence of intracellular cesium suggested tonic presynaptic GABA$_B$ inhibition. Tonic GABA$_B$ inhibition of uIPSPs originating from basket cells has been reported (Buhl et al. 1995). However, our results ruled out postsynaptic sites of actions and further suggest that tonic presynaptic GABA$_B$ inhibition of GABA release may be generalized to many types of interneurons.

**Fig. 6.** Tonic presynaptic inhibition of uIPSCs by GABA$_B$ receptors. A–C: single traces (left) and averaged uIPSCs (right) evoked by stimulation of a PYR interneuron and recorded with cesium methanesulfonate intracellular solution. After bath application of 5 μM CGP 55845A, individual and averaged uIPSCs were larger in amplitude (B) compared with control (A). The effect of the antagonist was partially reversible after 20 min of washout (C).

**Fig. 7.** Amplitude distribution of uIPSCs in CGP 55845A. A: cumulative probability distribution of uIPSC amplitude in control (solid) and in CGP 55845A (dotted). Number of events was 70 and 56, respectively, in each condition. The distribution was significantly shifted toward higher values in the GABA$_B$ antagonist. B: histograms of uIPSC amplitude distribution in control (top) and CGP 55845A (bottom). Although smaller events appeared similar in amplitude in both conditions, the amplitude of larger uIPSCs was greater in CGP 55845A (>100 pA) than in control (<100 pA).
Paired-pulse stimulation did not produce inhibition of uIPSCs, in contrast to the effects on compound monosynaptic IPSCs (Davies et al. 1990). This discrepancy may be due to the different stimulation protocols used. Others have also reported that fast IPSCs evoked by minimal stimulation in PYR do not show paired pulse depression within the 100- to 200-ms range, but that slow IPSCs evoked by minimal stimulation in stratum lacunosum-moleculare display...
GABA<sub>B</sub>-dependent paired pulse depression (Pearce et al. 1995). In the present study, only uIPSCs evoked by stimulation of interneurons in PYR and OR were tested with paired pulse stimulation. These uIPSCs may therefore correspond to the fast IPSCs that do not show paired pulse inhibition (Pearce 1993; Pearce et al. 1995). GABA<sub>B</sub>-independent paired pulse depression has also been described for IPSCs in hippocampus (Lambert and Wilson 1994; Pearce et al. 1995; Wilcox and Dichter 1994). This paired pulse depression is dependent on the probability of release during the first response (Lambert and Wilson 1994; Wilcox and Dichter 1994). Manipulations that reduced release probability decreased paired pulse depression and resulted in paired pulse facilitation (Lambert and Wilson 1994; Wilcox and Dichter 1994). In the present work, no significant paired pulse inhibition was observed. Individual inhibitory interneurons in the CA1 area can make many synaptic contacts (up to 12) with a single pyramidal cell (Buhl et al. 1994a), and these may not all be active each time a presynaptic cell is stimulated. In our experimental conditions, release probability for individual interneurons may have been low, because uIPSCs showed large fluctuations in amplitude with occasional transmission failures. In addition, the presence of tonic presynaptic GABA<sub>B</sub> inhibition likely contributed to reducing release probability. Thus differences in release probability under the various experimental conditions of each study may explain the discrepancy between our results and those of others (Lambert and Wilson 1994; Wilcox and Dichter 1994). Paired pulse stimulation of individual interneurons during manipulations that increase probability of release should help resolve this issue.

The origin of GABA responsible for tonic presynaptic inhibition remains unidentified. A heterosynaptic origin of GABA<sub>B</sub> inhibition is compatible with non-GABA<sub>B</sub>-mediated presynaptic autoinhibition at GABA synapses in hippocampal cell cultures (Wilcox and Dichter 1994; Yoon and Rothman 1991). Tonic presynaptic GABA<sub>B</sub> inhibition was not observed in some cells (see also Buhl et al. 1995), and presynaptic GABA<sub>B</sub> inhibition may be selective for distinct subsets of inhibitory fibers (Lambert and Wilson 1993; Pearce et al. 1995). Thus heterosynaptic GABA<sub>B</sub> mechanisms may inhibit GABA release only in subsets of interneurons. The functional consequences of such complex presynaptic modulation of inhibitory cells remain unclear, yet it points to a tremendous flexibility of local inhibitory processes in the hippocampus.

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UNITARY IPSCS IN CA1 PYRAMIDAL CELLS


