Novel glutamate- and GABA-independent synaptic depolarization in granule cells of guinea-pig hippocampus

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1. Dual intracellular recordings of granule cells, hilar interneurons and CA3 pyramidal cells were performed in transverse slices of guinea-pig hippocampus. At resting membrane potential, in the presence of 4-aminopyridine, ionotropic glutamate receptor antagonists and the $GABA_A$ receptor antagonist bicuculline, granule cells showed spontaneous, large amplitude depolarizations correlated with synchronous bursting activity of interneurons.

2. Under these conditions, pyramidal cells exhibited large amplitude monophasic $GABA_B$ inhibitory postsynaptic potentials (IPSPs) synchronous with the GABAergic interneuron burst discharges. The granule cells also received a $GABA_B$ input, which was evident only when the neurons were depolarized by DC injection. The $GABA_B$ receptor antagonist CGP 55845A (CGP) blocked the $GABA_B$ IPSPs in both pyramidal cells and granule cells; however, the depolarizing potential in granule cells was unaffected by the drug.

3. The granule cell depolarization in the presence of CGP was monophasic and exhibited linear voltage dependence with a reversal potential around −40 mV, suggesting that it was generated by a synaptic input activating a mixed cationic current.

4. The granule cell depolarization was abolished following the addition of tetrodotoxin to the bath. In addition, perfusing the slice with a low Ca^{2+}-containing solution (0·5 mM Ca^{2+}–10 mM Mg^{2+}) also abolished the granule cell depolarization, confirming the synaptic origin of the event.

5. (*S*)-Methyl-4-carboxyphenylglycine, L-(+)-2-amino-3-phosphonopropionic acid, propranolol and atropine did not affect the granule cell depolarization, indicating that metabotropic glutamate receptors, β-adrenergic receptors and muscarinic cholinergic receptors were not involved in generating the granule cell depolarizing synaptic response.

6. These findings indicate that, in the absence of both glutamatergic and GABAergic inputs, synchronous interneuronal activity can produce a depolarizing synaptic response in granule cells. The neurochemical responsible for the depolarization is currently under investigation.

Within the hippocampus, the trisynaptic circuit plays an essential role in the transfer of information. The dentate gyrus receives input from the entorhinal cortex via the perforant path and relays this information to CA3 pyramidal cells, which in turn activate CA1 pyramidal cells (for review, see Amaral & Witter, 1989). Thus, the dentate gyrus, as the initial entry point into the hippocampal formation, plays a crucial role in the gating of information transfer (Collins, Tearse & Lothman, 1983; Stringer, Williamson & Lothman, 1989).

Novel mechanisms of excitation to facilitate synaptic transmission have begun to be uncovered in the hippocampus. Within the dentate gyrus, these include recurrent connections among interneurons utilizing GABA as a depolarizing neurotransmitter (Michelson & Wong, 1991), and presumed gap junctions among interneurons (Michelson & Wong, 1994; Strata, Atzori, Molnar, Ugolini, Tempia & Cherubini, 1997; M. Forti & H. B. Michelson, unpublished observations). Some of these excitatory processes have been elucidated using the convulsant compound 4-aminopyridine (4-AP). Synchronization of interneuron firing by 4-AP, in the absence of ionotropic glutamate receptor-mediated activity, produces large amplitude inhibitory postsynaptic potentials (IPSPs) in pyramidal cells, consisting of a hyperpolarizing $GABA_A$ input, a depolarizing $GABA_A$ input, and a hyperpolarizing $GABA_B$ input (Michelson & Wong, 1991, 1994). Blockade of these

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GABA-mediated responses with bicuculline and CGP 55845A eliminates all synaptic events in pyramidal neurons which occur in the presence of 4-AP and ionotropic glutamate receptor blockers.

Under similar experimental conditions, however, we found that dentate granule cells behaved quite differently from pyramidal cells. In the presence of 4-AP and ionotropic glutamate receptor blockers, granule cells also exhibited enhanced GABAergic synaptic inputs. However, blockade of all $GABA_A$ and $GABA_B$-mediated events revealed a depolarizing potential, heretofore never described in granule cells, which does not exist in pyramidal cells. The following report provides the first detailed description of this glutamate-independent, GABA-independent depolarizing event in dentate granule cells.

METHODS

Transverse slices of hippocampus were prepared from 14- to 21-day-old male guinea-pigs using standard procedures in accordance with guidelines set by the local animal care review committee. Briefly, animals were rapidly decapitated after being anaesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane by inhalation. After isolation of the hippocampus from the whole brain, 400 μm thick slices were cut with a Vibratome Series 1000 and transferred onto the nylon mesh of a gas–fluid interface recording chamber. The chamber was maintained at 34–35 °C, at a pH of 7·4, and exposed to a warm, humidified atmosphere saturated with a 95% O_2–5% CO_2 gas mixture. The perfusion solution contained NaCl, 124 mM; KCl, 5 mM; $CaCl_2$, 2 mM; $MgCl_2$, 1·6 mM; $NaHCO_3$, 26 mM; D-glucose, 10 mM; 4-aminopyridine (4-AP), 75–100 μM; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 20 μM; 3-3(2-carboxypiperazine-4-yl)propyl-1-phosphonate (CPP), 20 μM and bicuculline methiodide, 10–15 μM. In some experiments, slices were bathed in a low Ca^{2+}-containing solution (0·5 mM Ca^{2+}–10 mM Mg^{2+}) in order to block synaptic transmission (Aram, Michelson & Wong, 1991). CGP 55845A (CGP), 20 μM; (*S*)-methyl-4-carboxyphenylglycine (MCPG), 1–10 mM; L-(+)-2-amino-3-phosphonopropionic acid (L-AP3), 5–10 mM; propranolol, 20 μM; tetrodotoxin (TTX), 0·6 μM or atropine, 10 μM were added to the solution in some experiments. CNQX, CPP, L-AP3 and MCPG were obtained from Tocris Cookson and CGP 55845A was kindly provided by Ciba-Geigy. All other chemicals were obtained from Sigma Chemical Company.

Dual intracellular recordings were performed using glass micropipettes (40–80 MΩ) filled with 3 M potassium acetate or with 2% (w/v) biocytin in 1 M potassium acetate for granule cell and interneuron staining. Only one granule cell or interneuron per slice was recorded in order to avoid multiple intracellular staining. In some experiments, the micropipettes were filled with lidocaine *N*-ethyl bromide (80 mM in 3 M potassium acetate) in order to block sodium spikes. Signals were amplified by a dual channel Neurodata amplifier and stored on tape for later off-line analysis.

At the end of the experiments, the slices were fixed overnight with 4% (v/v) paraformaldehyde for biocytin processing. The slices were incubated in ABC complex (Vectastain, Vector Laboratories) and were processed with diaminobenzidine for the visualization of the stained cells. The slices were dehydrated in a series of alcohol rinses before being examined with a microscope having appropriate photographic accessories.

RESULTS

Simultaneous dual recordings of granule cells and CA3 pyramidal cells ($n = 12$) or of hilar interneurons and pyramidal cells ($n = 3$) were performed with 4-AP, CNQX and CPP in normal perfusion solution. All cells were filled with biocytin for later morphological identification. Under these conditions, spontaneous synchronous burst discharges of GABAergic interneurons occur in the absence of glutamatergic excitation, with each interneuron burst correlating with a synchronized IPSP in pyramidal cells (also see Aram *et al.* 1991; Michelson & Wong, 1991, 1994).

In addition to the ionotropic glutamate receptor blockers, bicuculline (10–15 μM) was added to the perfusion solution in the present experiments to block $GABA_A$ receptor-dependent events. Under these experimental conditions, only some subtypes of hilar interneurons exhibited spontaneous bursting activity, which was correlated with synchronous monophasic $GABA_B$ receptor-mediated IPSPs in pyramidal cells (Fig. 1*A*; see also Michelson & Wong, 1994; M. Forti & H. B. Michelson, unpublished observations). In contrast, under identical conditions at resting membrane potential (RMP; $-72{\cdot}6 \pm 3{\cdot}7$ mV; mean ± S.D.), all granule cells exhibited large amplitude spontaneous depolarizations ($8{\cdot}4 \pm 1{\cdot}2$ mV) at a frequency of 0·06–0·14 Hz, which occurred synchronously with each pyramidal cell $GABA_B$ IPSP (Fig. 2*A*). Similar synchronized depolarizing responses in granule cells could also be elicited by extracellular stimulation of the hilar region with bipolar tungsten electrodes (100 μs, 0·2–0·3 mA pulses). The granule cell depolarizations also occurred simultaneously with the interneuron depolarizations ($n = 2$; not shown). Since the pyramidal cell IPSPs were observed to occur synchronously with both the granule cell depolarizations and the interneuron bursts, it is clear that the spontaneous synchronized events were occurring simultaneously in all three cell populations.

When the granule cells were depolarized with DC, the large amplitude synchronous depolarizations clearly appeared to be composed of an overlapping depolarizing and hyperpolarizing component (Fig. 1*B*, right). The hyperpolarizing component had a reversal potential around −90 mV, similar to the monophasic hyperpolarization in pyramidal cells and was attributed to a $GABA_B$ input (see below). The depolarizing component had a reversal potential more positive than −50 mV and was hypothesized to be generated by a synaptic input which was not mediated by ionotropic glutamate receptors or by $GABA_A$ receptors.

When CGP (20 μM) was added to the perfusion solution, the $GABA_B$ input was blocked in both pyramidal cells and granule cells ($n = 5$; Fig. 2*A*, CGP). However, the large depolarizing events continued to occur spontaneously in granule cells. Thus, CGP blocked all the remaining synchronized events in pyramidal cells, but not in granule cells, where the synchronized events persisted and could therefore be only partially attributed to the $GABA_B$ input.

The balance between the depolarizing input and the $GABA_B$ input was critical in determining the response of the granule cells during the spontaneous large amplitude events. In granule cells with similar RMPs, when the $GABA_B$ inhibitory input was strong ($n = 6$) the depolarization did not reach the threshold for a burst. In some cases, however, the $GABA_B$ inhibitory input was comparatively weaker ($n = 2$), and granule cells exhibited spontaneous bursting activity similar to that observed in interneurons (see Fig. 6). After CGP wash-in, the depolarizations in the granule cells became monophasic and large enough to elicit a burst discharge in cells that had not reached threshold previously (Fig. 2*B*).

In order to characterize the synchronized depolarization, two granule cells were recorded with electrodes containing lidocaine *N*-ethyl bromide (80 mM) to block sodium spike generation. The granule cell depolarization was isolated by blocking the $GABA_B$ response with CGP, and the granule cells were hyperpolarized and depolarized by DC injection in order to calculate the reversal potential of the depolarization. In Fig. 3, the amplitude of the depolarization in a biocytin-stained granule cell was measured at different membrane potentials and plotted against the corresponding membrane potential. The latency of the measurements was fixed at the peak of the depolarization in the most hyperpolarized trace

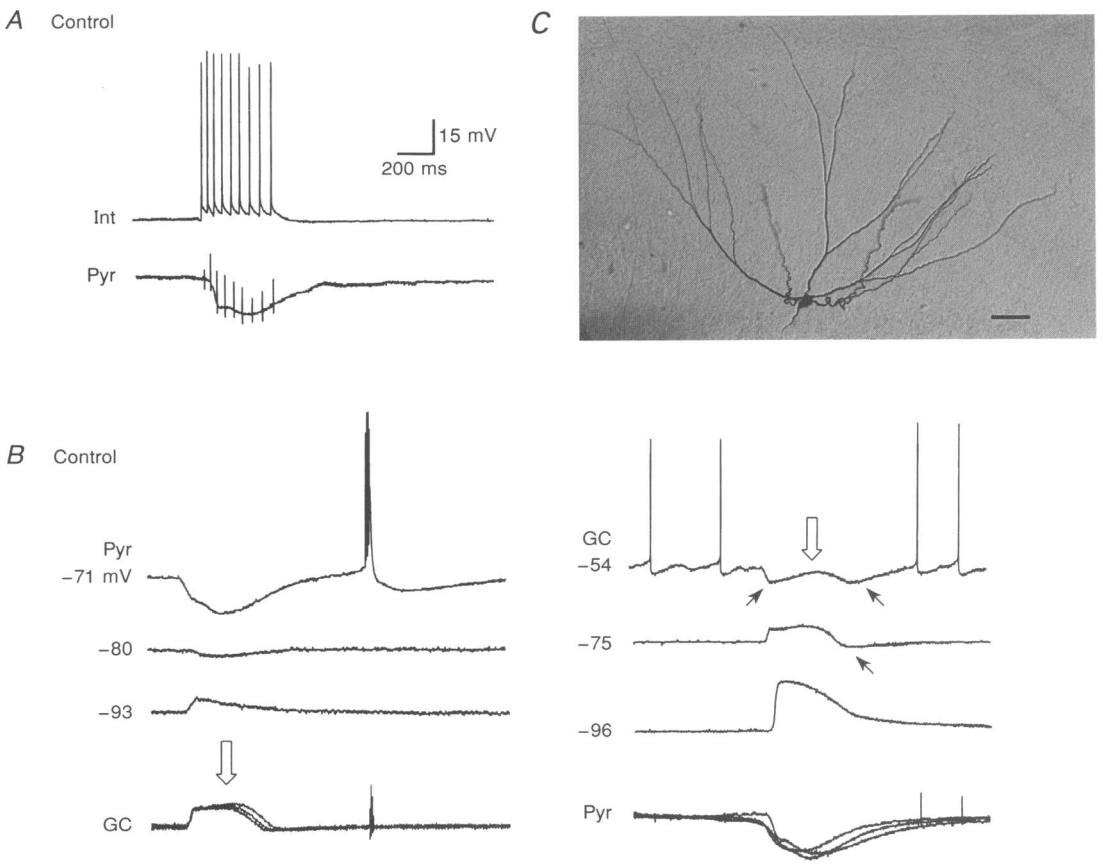

Figure 1. Interneuron spontaneous discharge, pyramidal cell synchronous IPSP and granule cell synchronous depolarization

A, spontaneous bursting activity in a hilar interneuron (Int) and synchronous $GABA_B$ IPSP recorded in a CA3 pyramidal cell (Pyr) in the presence of 4-AP (100 μM), CNQX (20 μM), CPP (20 μM) and bicuculline (15 μM) (Control). Electrical capacitance transients associated with the firing of the simultaneously recorded interneuron are visible in the pyramidal cell trace. Similar artifacts are present in all the other dual recordings. *B*, simultaneous recordings of a granule cell (GC) and a CA3 pyramidal cell; resting membrane potentials (RMPs) were −75 and −71 mV, respectively. Spontaneous events in control conditions were recorded at different membrane potentials in the pyramidal cell (left traces) or in the granule cell (right traces) while the other cell was left at rest to monitor the frequency and consistency of repetitive events. Note that the large amplitude spontaneous events in the pyramidal cell are monophasic and have a reversal potential between −80 and −93 mV, whereas the synchronous large amplitude events in the granule cell are biphasic potentials, having a depolarizing component (open arrow) which does not revert at any of the shown potentials, and a hyperpolarizing component (filled arrows) with a reversal potential between −75 and −96 mV. *C*, photograph (×20 objective lens) of the granule cell in *B* which was processed for biocytin visualization after the recording. Calibration bar, 32 μm.

in order to avoid the contamination of late voltage-dependent currents that might be activated by the depolarization. The depolarization exhibited a linear voltage dependence with a reversal potential of −42 mV, suggesting it was generated by a synaptic input that might activate a mixed cationic current. Similar results were obtained in the other cell. These findings indicate that granule cells receive an excitatory synaptic input which is independent of ionotropic glutamate receptor and GABA receptor activation.

The synaptic origin of the granule cell depolarization was confirmed when the sodium channel blocker TTX (0·6 μM) was added to the perfusion solution. TTX abolished the granule cell depolarization, indicating that presynaptic firing is necessary for the generation of the event (Fig. 4*A*). When slices were bathed in a low Ca^{2+} (0·5 mM Ca^{2+}–10 mM Mg^{2+}) perfusion solution to eliminate synaptic transmission, the granule cell depolarizations were reversibly blocked (Fig. 4*B*), further confirming that the events were generated by a synaptic mechanism.

The nature of the excitatory input was examined by adding various antagonists to the perfusion solution in an attempt to block the granule cell depolarization. MCPG (10 mM), L-AP3 (10 mM), propranolol (10 μM) and atropine (10 μM) had no effect on the granule cell depolarization, indicating that metabotropic glutamate receptors, β-adrenergic receptors and muscarinic cholinergic receptors were not involved in the generation of the granule cell excitatory synaptic response (Fig. 5).

The physiological significance of the granule cell depolarization was investigated by monitoring the spontaneous excitatory events before and after blockade of $GABA_A$ and $GABA_B$-mediated responses ($n = 6$). Before the block of $GABA_A$ inputs with bicuculline, the granule cell depolarization was larger than after bicuculline wash-in, due

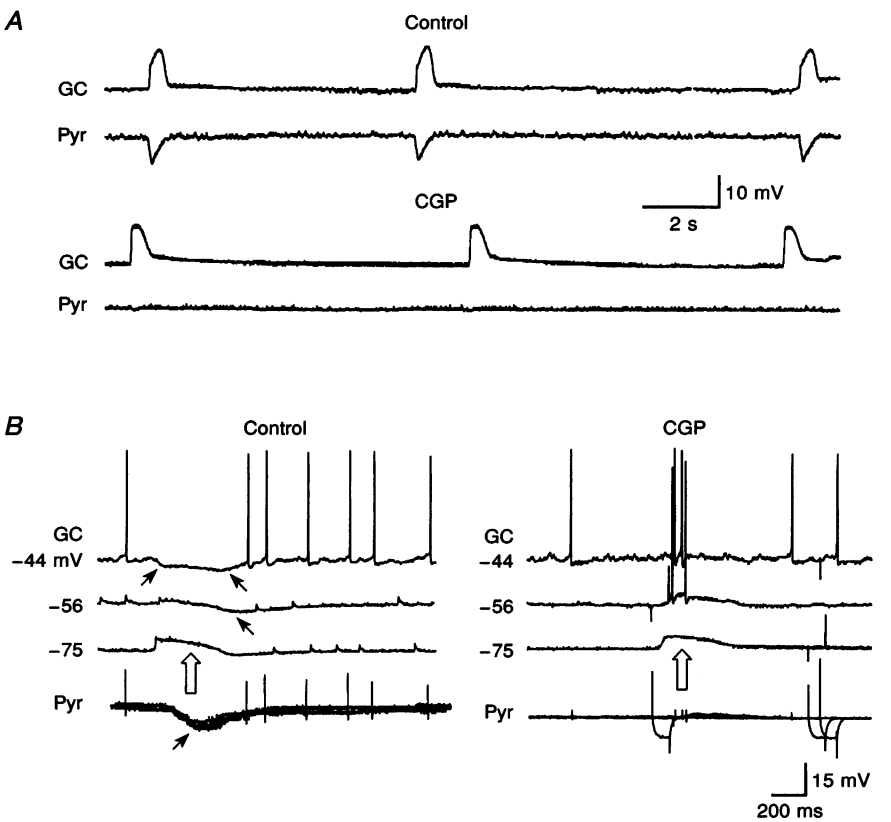

Figure 2. $GABA_B$ receptor blockers abolish the synchronous IPSPs in pyramidal cells but not the synchronous depolarizations in granule cells

A, spontaneous activity in a dual recording of a granule cell and a pyramidal cell before (top) and after (bottom) CGP 55845A (CGP; 20 μM) addition to the perfusion solution. RMPs were −75 mV for the granule cell and −66 mV for the pyramidal cell and remained constant throughout the experiment. Note that the spontaneous activity was blocked by CGP in pyramidal cells, but not in granule cells. *B*, the granule cell spontaneous events in a different experiment were recorded at different membrane potentials to show the effect of CGP on the hyperpolarizing input (filled arrows). Intracellular hyperpolarizing pulses were injected into the pyramidal cell to verify the stability of the cell impalement. Note the block of the $GABA_B$ input in both the pyramidal cell and the granule cell and the CGP-resistant monophasic depolarization (open arrow) in the granule cell.

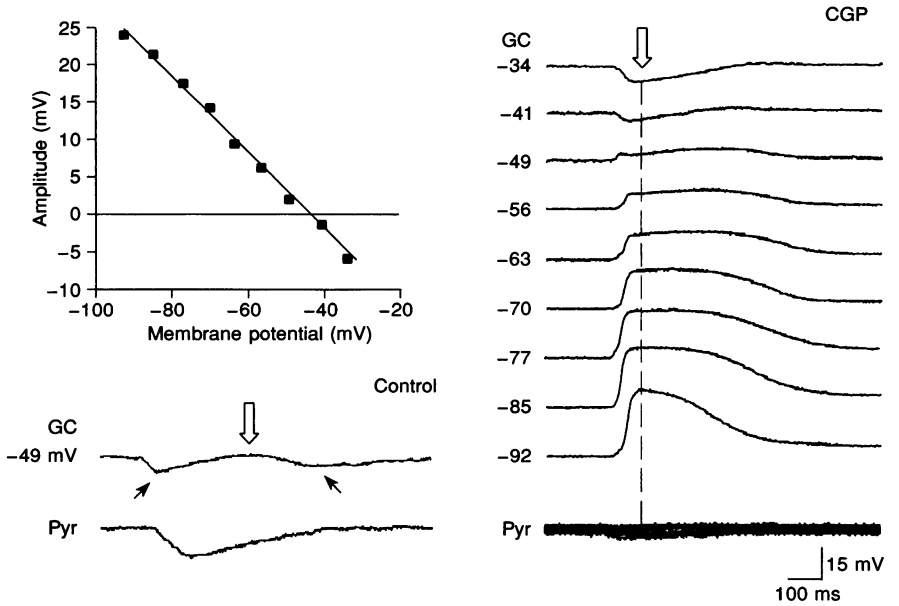

Figure 3. Reversal potential of the granule cell synchronous depolarization

A granule cell was recorded with an electrode containing lidocaine *N*-ethyl bromide at 80 mM before (Control, lower left) and after block of the $GABA_B$ input by CGP (right). The filled arrows in the lower left traces indicate the hyperpolarizing $GABA_B$ input. RMP was −70 mV and input resistance was 24 MΩ. The amplitude of the monophasic depolarization in the presence of CGP was measured at different membrane potentials in order to calculate its reversal potential. DC injection was from −0·9 to +1·6 nA. The amplitudes of the spontaneous events were measured at the positive peak in the most hyperpolarized trace (right, vertical dashed line) and plotted against the membrane potential (top left). The reversal potential of the granule cell synchronous depolarization (open arrow) was −42 mV.

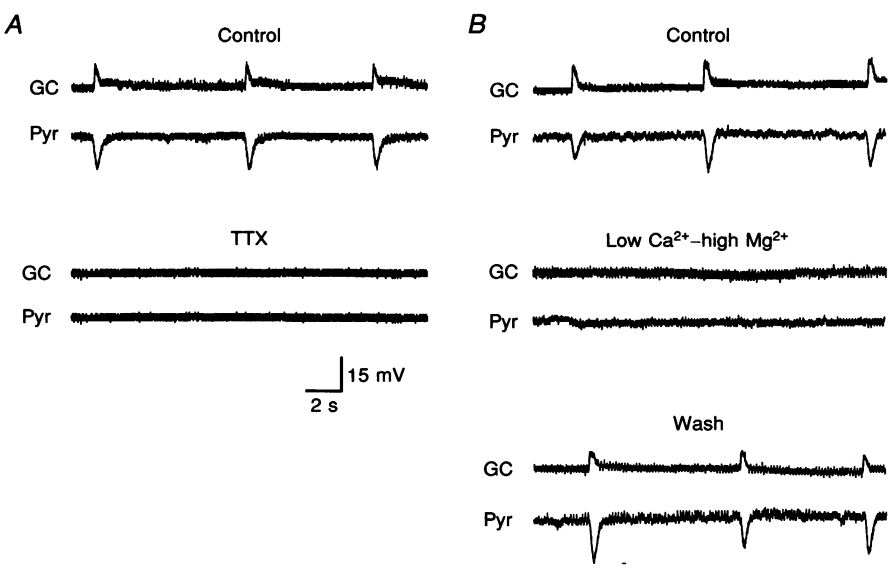

Figure 4. TTX and low calcium block the granule cell depolarization

A, TTX (0·6 μM) administration during a dual recording of a granule cell and a pyramidal cell. RMPs were −73 and −63 mV, respectively, and did not change during the course of the experiment. Note the block of synaptic events in both cells. *B*, effect of perfusion of a low Ca^{2+}–high Mg^{2+}-containing solution (0·5 mM Ca^{2+}–10 mM Mg^{2+}) in a different experiment. Granule cell RMP was −72 mV; pyramidal cell RMP was −65 mV. Note the reversible block of the synaptic events in both cells.

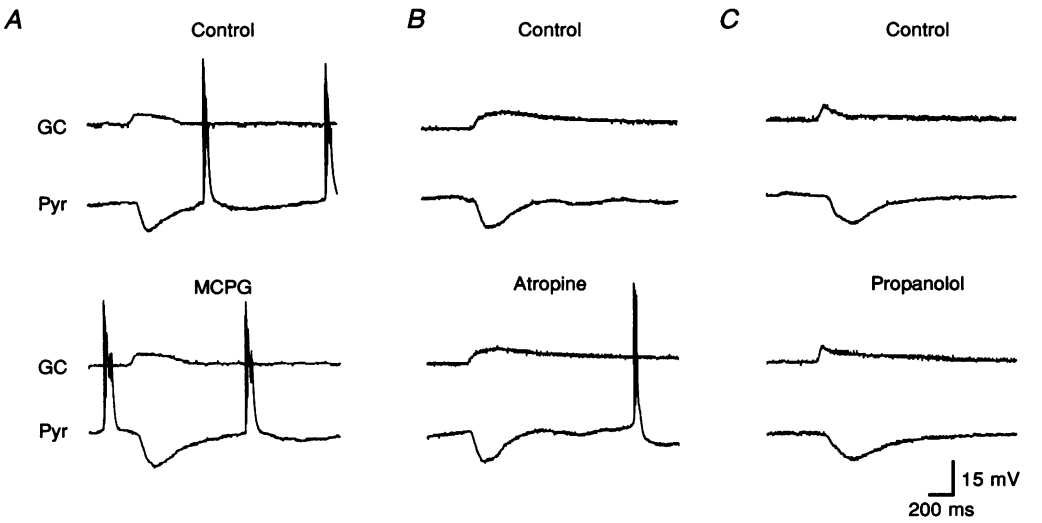

Figure 5. The granule cell depolarization is resistant to MCPG, atropine and propranolol
Administration of MCPG (10 mM), atropine (10 μM) and propranolol (10 μM) in three different granule cells. The RMPs were −76 mV in *A*, −74 mV in *B* and −73 mV in *C*, and did not change during the experiment. Note that the spontaneous events are not significantly affected by the drugs.

to a significant $GABA_A$ depolarizing input. Under these conditions five out of six cells reached threshold generating a burst. After adding bicuculline to the perfusion solution, two cells continued to burst (Fig. 6), whereas, in the others, the depolarization was below firing threshold. In the two cells which exhibited bursting activity after bicuculline wash-in, CGP did not significantly alter the spontaneous bursts. In contrast, in the other granule cells following the blockade of $GABA_B$ inhibition with CGP, the cells resumed their bursting activity at or close to the resting membrane potential (see Fig. 2). This indicated that in the presence of 4-AP, when GABA receptor activation was not altered, the GABA-independent depolarization in granule cells in combination with the $GABA_A$ depolarizing input was strong enough to override the hyperpolarizing $GABA_A$ and $GABA_B$ inputs. This finding suggests that, in our experimental model of hippocampal hyperexcitability, the input of GABAergic interneurons onto granule cells is mainly excitatory and can elicit burst firing in these cells.

DISCUSSION

The results of the present study indicate that dentate granule cells receive an excitatory synaptic input which is not generated by either glutamatergic or GABAergic receptor activation. In contrast to granule cells, pyramidal cells exhibit no synaptic activity following ionotropic glutamate and GABA receptor blockade. The synaptic nature of the granule cell depolarization was demonstrated by its linear voltage dependence. In addition, the depolarization was abolished in conditions in which synaptic transmission is blocked, such as following TTX administration or after

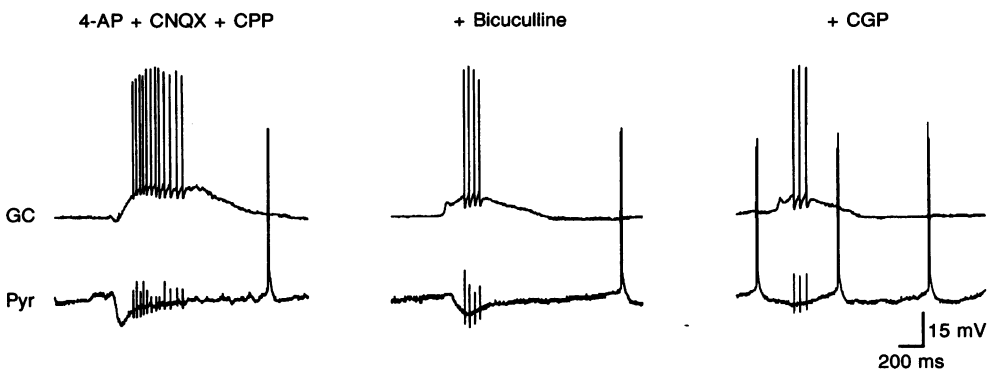

Figure 6. Granule cell depolarization overrides GABAergic inhibition
Effect of 15 μM bicuculline and 20 μM CGP on the granule cell bursting activity sustained by the synchronous depolarizing input. RMP of the granule cell was −77 mV. Note that the burst length is reduced by bicuculline due to the block of the $GABA_A$ depolarizing input, whereas it is not significantly affected by the block of the $GABA_B$ input. Concentrations of 4-AP, CNQX and CPP used are as in Fig. 1.

perfusion with a low Ca^{2+}-containing solution, further confirming its synaptic origin.

Previous studies demonstrated that, in our experimental protocol, only certain morphologically identified interneurons with specific intrinsic properties and connectivity exhibited bicuculline-resistant bursts corresponding to the synchronized $GABA_B$ responses in pyramidal cells (Michelson & Wong, 1994; M. Forti & H. B. Michelson, unpublished observations). These interneurons, which continued to burst after the $GABA_B$ receptor blocker CGP was administered, were identified as spheroid interneurons of the dentate–hilus (D–H) border and oviform interneurons (Amaral, 1978). Other subpopulations of hilar interneurons, as well as hilar mossy cells, were either synaptically silent in these conditions or exhibited synchronized $GABA_B$ IPSPs, similar to pyramidal cells. Therefore, it is most likely that either spheroid or oviform interneurons, or both subpopulations, are the cells generating the synchronous depolarization in the granule cells. This would be the first evidence that hilar inhibitory cells can generate GABA-independent excitatory events in granule cells.

Spheroid interneurons of the D–H border were also found to exhibit a GABA- and glutamate-independent synaptic depolarization (M. Forti & H. B. Michelson, unpublished observations). It is possible that the non-glutamate, non-GABA-mediated synaptic depolarizations observed in granule cells and in spheroid interneurons are generated by the same mechanism. The granule cell depolarization had a reversal potential of approximately −40 mV, suggesting that a mixed cationic current or different second messenger-activated currents might be involved in the postsynaptic response. Although the receptors involved in the response have yet to be identified, metabotropic glutamate receptors, β-adrenergic receptors and muscarinic cholinergic receptors should be excluded, as MCPG, L-AP3, propranolol and atropine did not block the granule cell depolarization.

Because dentate granule cells are the initial informational point of entry in the trisynaptic circuit, they play a critical role in processing and gating synaptic neurotransmission into the hippocampus (Stringer & Lothman, 1989). In this light, many investigators have noted that dentate granule cells are more resistant to generating repetitive firing (Dudek, Deadwyler, Cotman & Lynch, 1976; Fournier & Crepel, 1984; Pan & Stringer, 1996), and may act to filter out high frequency discharges from the entorhinal cortex (Stringer *et al.* 1989). However, granule cell discharge is a crucial event in the development of ictal-like events in the hippocampal–entorhinal circuit (Stringer *et al.* 1989; Paré, de Curtis & Llinàs, 1992). Dentate granule cells have been found to burst synchronously in other models of neuronal hyperexcitability (Scharfman, 1994; Schweitzer & Williamson, 1995). Indeed, in the presence of elevated extracellular potassium, granule cells can burst in the absence of amino acid neurotransmission (Schweitzer & Williamson, 1995). Some investigators have suggested that non-synaptic mechanisms contribute to the synchronous burst firing in dentate granule cells (Schweitzer & Williamson, 1995; Pan & Stringer, 1996). We have previously demonstrated that elevating the extracellular potassium concentration will synchronize interneuronal firing (Michelson & Wong, 1991). Thus it seems likely that the interneuron-generated depolarizing synaptic potential described in the present study with 4-AP would also contribute to the synchronization of granule cell bursting activity described in other models.

In conclusion, our results demonstrate that the glutamate- and GABA-independent synaptic depolarization in granule cells is strong enough to elicit a burst discharge, particularly when added to the $GABA_A$-mediated depolarizing input received by the cells in the absence of bicuculline. This implies that in conditions of increased excitability, when synchronized interneuronal activity is generated, the input of the hilar interneurons onto granule cells would be excitatory instead of inhibitory, thus facilitating burst firing in granule cells. Although the precise physiological role of this non-glutamate, non-GABA-mediated synaptic depolarization is not known at this time, it is likely that, by virtue of its ability to override GABAergic inhibition in granule cells, it plays an important role in modulating synaptic transmission within the hippocampus.

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