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Paradoxical enhancement by bicuculline of dentate granule cell IPSPs evoked by fimbria stimulation in rat hippocampal slices

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Abstract

Stimulation of the fimbria in rat hippocampal slices evoked an extracellular negativity in the granule cell layer and a small depolarization in granule cells at their resting potentials. The intracellular potentials appeared to be $GABA_A$ receptor-mediated IPSPs because they reversed at −69.1 ± 1.0 mV (mean ± S.E.M.. n = 14) and were blocked by the $GABA_A$ receptor antagonist bicuculline (10–50 μM, n = 14). However, during the first few minutes of perfusion with bicuculline. IPSPs transiently and paradoxically *increased* in amplitude. As IPSPs increased, the reversal potential and latency to onset remained the same. These effects were reversible, and during the wash period IPSPs first increased and then stabilized at a smaller amplitude, similar to IPSPs evoked in control conditions. As the $GABA_A$ receptor-mediated IPSP decreased, it was followed by a second hyperpolarization. This late hyperpolarization appeared to be a $GABA_B$ receptor-mediated IPSP, because it reversed near the equilibrium potential for potassium (mean −81.8 ± 2.3 mV, n = 12, $[K^+]_o$ = 5 mM) and was blocked by the $GABA_B$ receptor antagonist 2-hydroxy saclofen (250–500 μM, n = 5). The results suggest that $GABA_A$ and $GABA_B$ receptor-mediated IPSPs evoked in granule cells by fimbria stimulation are normally inhibited by activation of $GABA_A$ receptors. The inhibition by $GABA_A$ receptors is strong enough that, in control conditions, the $GABA_A$ IPSPs are barely detectable and the $GABA_B$ IPSPs are undetectable. The relevant $GABA_A$ receptors could be located presynaptically, on the nerve terminals of inhibitory interneurons that innervate granule cells, or on the dendrites and somata of the interneurons, where they may be affected by GABAergic inputs activated by fimbria stimulation. These data demonstrate the strength and complexity of pathways utilizing $GABA_A$ receptors and $GABA_B$ receptors to inhibit dentate granule cells.

Keywords

IPSP; GABA; Presynaptic inhibition; $GABA_A$ receptor; $GABA_B$ receptor; Dentate gyrus; Septohippocampal projection; Interneuron

The control of hippocampal inhibition is of great importance, given the evidence that inhibition controls hippocampal excitability [8,17,29,32] and affects synaptic plasticity [7,21,34]. Inhibition in the dentate gyrus may be particularly important in controlling the spread of epileptic discharges through the limbic system [25]. Numerous studies have examined the nature and control of hippocampal inhibition, and a complex set of mechanisms has emerged (for review see ref. 1). One source of control is exerted by

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presynaptic $GABA_B$ receptors that suppress the release of GABA in hippocampus, [3,6,9,11,12,33] as well as the dentate gyrus [4,18,20,22]. In addition, some studies have indicated that presynaptic $GABA_A$ receptors, i.e., $GABA_A$ receptors on GABAergic interneurons, may modulate $GABA_A$ receptor-mediated inhibition. For example, it has been reported that the $GABA_A$ receptor antagonist picrotoxin blocks frequency-dependent depression of IPSPs in area CA1, [2] and that the $GABA_A$ agonist muscimol disinhibits immature CA1 pyramidal cells [5]. There is a precedent for presynaptic inhibition by $GABA_A$ receptors, since it has been shown in rat frontal cortex that $GABA_A$ receptors are responsible for presynaptic inhibition of GABA release [19]. The results presented below describe (1) a $GABA_A$ and $GABA_B$ receptor-mediated IPSP evoked in granule cells by fimbria stimulation, and (2) strong inhibition of that IPSP that is mediated by $GABA_A$ receptors.

Transverse rat hippocampal slices were prepared as previously described [26]. Animals were treated in accordance with guidelines set by the National Institutes of Health and the New York State Department of Health. Briefly, adult female Sprague–Dawley rats were anesthetized with ether and decapitated. The brain was immediately removed and the hippocampus was isolated within a block of tissue including adjacent structures. The hippocampus was cut in 400 μm thick slices with a vibratome while immersed in 4°C buffer (in mM: 126.0 NaCl, 5.0 KC1, 2.0 $CaCl_2$, 2.0 $MgCl_2$, 26.0 $NaHCO_3$, 1.25 NaH_2PO_4, and 10.0 D-glucose; pH 7.4). Slices were immediately transferred to a recording chamber (Fine Science Tools), where they were perfused with warm (33–34°C), oxygenated (95% O_2, 5% CO_2) buffer.

Recordings were made with glass microelectrodes (borosilicate glass containing a capillary fiber, A&M Systems) that were filled with 1 M potassium acetate (80–150 MΩ), using an intracellular amplifier with a bridge circuit (Axoclamp 2A, Axon Instruments); bridge balance was monitored continuously. For stimulation of the fimbria, a twisted metal bipolar electrode was placed in the white matter of the fimbria (Fig. 1). Fimbria stimulation was set at the level required to evoke a submax-imal response of CA3b pyramidal cells in the same slice; any slice without at least a 5 mV antidromic population spike in CA3b was not used. Data were recorded on an oscilloscope (Nicolet) and taped (Neurodata Instruments) for analysis offline. Bicuculline methiodide (10 mM in 0.9% NaCl, Sigma), 2-hydroxy saclofen (10 mM in 10 mM NaOH, Tocris Neuramin), atropine meth-ylbromide (10 mM in 0.9% NaCl, Sigma), and mecam-ylamine (10 mM in 0.9% NaCl, Sigma) were stored in concentrated aliquots and dissolved in buffer to reach the desired final concentration immediately before use.

This study was based on intracellular recordings from 38 granule cells and extracellular recordings from 45 slices. Fimbria stimulation was used to evoke an IPSP in dentate granule cells without concomitant orthodromic or antidromic excitation, as occurs when IPSPs are elicited by perforant path input or mossy fiber stimulation. Fimbria-evoked IPSPs were detected both extracellularly as well as intracellularly. The extracellular correlate to the IPSP was a small negativity recorded in the granule cell layer (mean amplitude 0.5 ± 0.1 mV, n = 45) with a latency to onset of 9.6 ± 0.4 ms (Fig. 1). This negativity became smaller as the recording site was moved either towards the hilus or the molecular layer, and correlated with a positivity in the outer molecular layer (Fig. 1). It is unlikely that the positivity was passively conducted from stratum lacunosum-moleculare of area CA1, where a positivity was evoked by the same fimbria stimulus, because similar positivities in the molecular layer were recorded in the upper and lower blades (Fig. 1 A,B). The negativity in the granule cell layer was unaffected by bath-application of the cholinergic muscarinic antagonist atropine co-applied with the nicotinic antagonist me-camylamine (10 μM each, n = 4; 25 μM, n = 3; 50 μM, n = 2; data not shown).

The intracellularly-recorded IPSP was a small depolarization of granule cells at their resting potential, and a hyperpolarization at more depolarized potentials (Fig. 1). The hyperpolarizations were small (mean maximal hyperpolarization 2.9 ± 0.3 mV, *n* = 14; Fig. 1) and began at a mean latency of 10.1 ± 0.6 ms after the fimbria stimulus. The mean latencies of the intracellular and extracellular potentials were not significantly different (*t*-test, P > 0.05), supporting the premise that they were due to similar mechanisms. The reversal potentials of hyperpolarizations were close to the equilibrium potential for chloride (mean reversal potential, −69.1 ±1.0 mV, *n* = 14), indicating that they were IPSPs mediated by GABA acting at $GABA_A$ receptors. Consistent with that possibility, bicuculline blocked the IPSP in every cell where it was tested (Fig. 2, 10 μM, *n* = 2; 25 μM, *n* = 7; 50 μM, *n* = 5). Atropine and mecamylamine did not affect the IPSP (n = 9; data not shown).

During the first few minutes of bicuculline application, a transient increase in the amplitude of the IPSP occurred in all experiments (*n*= 14; Fig. 2). At the point during bicuculline application when IPSPs were largest, maximum IPSP amplitude was 7.0 ± 0.5 mV, 241% of the mean amplitude of control IPSPs. The latencies to onset of the IPSPs did not change during bicuculline bath-application (post bicuculline, 10.0 ±0.5 ms, *n* = 14, paired *t*-test, *P* > 0.05). The reversal potential of the increased IPSP was not different from the IPSP evoked in control conditions (mean E_{rev} post bicuculline = −69.5 ± 0.8 mV, *n* = 14, paired *t*-test, *P* > 0.05). Input resistance did not change significantly (control, 65 ± 3.4 MΩ; post bicuculline, 72 ± 5.1 Ω, *n* = 14, paired *t*-test, *P* > 0.05). The effects of bicuculline were completely reversible. During reversal, the $GABA_A$ IPSP first became quite large and subsequently stabilized at the small amplitude of control IPSPs (Fig. 2).

During the blockade of the $GABA_A$ IPSP, a second distinct hyperpolarization emerged at a longer latency from the stimulus (Figs. 2 and 3). After the initial $GABA_A$ IPSP was blocked, only the second hyperpolarization was evident (Fig. 2). This hyperpolarization was large (mean maximum amplitude, 8.7 ± 1.0 mV, *n* = 12), long lasting (mean duration 986 ± 14 ms, peak at 150–280 ms after the stimulus) and reversed at −81.8 ± 2.3 mV (*n* = 12). Application of the $GABA_B$ receptor antagonist 2-hydroxy saclofen (250–500 μM, *n* = 5) blocked the late hyperpolarization reversibly (Fig. 3). A small depolarization remained after saclofen application in some cases (Fig. 3); investigation of this apparent EPSP is currently underway.

The results demonstrate that in control conditions fimbria stimulation evokes very small IPSPs in granule cells that are mediated by $GABA_A$ receptors. The GABAergic septohippocampal pathway could mediate this IPSP. However, the long latency to onset of the IPSP, and the evidence that GABAergic septohippocampal neurons innervate interneurons preferentially [10,15], suggest that another circuit is also possible. One possibility is that fimbria stimulation excited pyramidal cells that subsequently excited dentate interneurons, and the interneurons were responsible for the granule cell IPSPs. This pathway is suggested by drawings of intracellularly-la-belled CA3 pyramidal cells, which have axon collaterals in the hilus; [13,16] these collaterals could innervate dentate interneurons. Either hilar interneurons or interneu-rons in the granule cell layer could be responsible for granule cell IPSPs, since both have dendrites in the hilus and both inhibit granule cells [28]. This pathway is supported by several lines of evidence. First, a large antidromic (and hence short latency) pyramidal cell population spike was evoked by the same stimulus that evoked IPSPs in granule cells. Second, pyramidal cells are thought to use an excitatory amino acid as a neurotransmitter [23], and fimbria-evoked IPSPs were insensitive to cholinergic antagonists yet sensitive to the glutamatergic antagonist CNQX. In five experiments bath-application of 5 μM CNQX blocked fimbria-evoked IPSPs completely (data not shown). Blockade of fimbria-evoked IPSPs by CNQX was completely reversible in three of five granule cells where impalements were maintained for over 30 minutes after

returning to CNQX-free buffer. In the other two cells blockade of the IPSP was only partly reversed. One variant of this pathway involves the ability of hilar 'mossy' cells to substitute for the pyramidal cells. Thus, hilar mossy cells could be excited by fimbria stimulation and in turn excite inhibitory interneurons of the dentate gyrus. In support of the latter possibility, fimbria stimulation in slices can excite hilar mossy cells by a CNQX-sensitive mechanism [27], mossy cells are thought to use glutamate as a neurotransmitter [31], and it has been argued that mossy cells innervate inhibitory interneurons [30]. Thus either pyramidal cells or mossy cells, or both, could be responsible for the excitation of GABAergic neurons that caused fimbria-evoked IPSPs in granule cells.

That an IPSP evoked in a hippocampal principal cell is mediated by $GABA_A$ receptors is not surprising, given that IPSPs in the hippocampus and dentate gyrus involve, either partially or exclusively, $GABA_A$ receptors [1,9,14]. It also is not surprising that the extracellularly-recorded IPSP was maximal in the granule cell layer and reversed in the molecular layer, since the site of the inhibitory 'basket' cell plexus is in the granule cell layer/inner molecular layer [24]. However, it is notable that blockade of $GABA_A$ receptors can increase a $GABA_A$ receptor-mediated IPSP and uncover large $GABA_B$ receptor-mediated IPSPs.

There are several possible mechanisms that could explain how $GABA_A$ receptors inhibit $GABA_A$ receptor-mediated granule cell IPSPs. First, GABA may normally act to inhibit its own release by $GABA_A$ autoreceptors. If this were the case, bicuculline might have impaired presynaptic inhibition at a time when postsynaptic $GABA_A$ receptors were incompletely blocked, resulting in a transient increase in the $GABA_A$ IPSP of granule cells. Another possibility is that bicuculline disinhibited interneurons at a time when $GABA_A$ receptors on granule cells were only partially blocked. Indeed, it has been shown that fimbria stimulation can excite and inhibit dentate hilar interneurons [27], so that $GABA_A$ receptor blockade could result in enhanced excitement of interneurons by a fimbria stimulus.

The appearance of a $GABA_B$ IPSP following bicuculline bath-application can be explained by disinhibi-tion of inhibitory interneurons as well. However, other factors must be considered to explain the absence of the $GABA_B$ IPSP relative to the $GABA_A$ IPSP in control conditions. One possible explanation is that the interneurons that cause $GABA_B$ IPSPs are more strongly inhibited by fimbria stimulation than the interneurons that are responsible for $GABA_A$ IPSPs. A second explanation is that $GABA_B$ receptors occur at the same synapses where $GABA_A$ receptors exist, but because $GABA_B$ receptors are further from the active zone they require greater release of GABA for their activation. Finally, $GABA_B$ synapses may be located electrically distal to the intraso-matic electrode, a location that could make $GABA_B$ IPSPs undetected until large amounts of GABA were released. Further experiments will be necessary to differentiate among these possibilities, but regardless of the underlying mechanisms (s), the results underscore the strength and complexity of the inhibitory network in the hippocampus and dentate gyrus.

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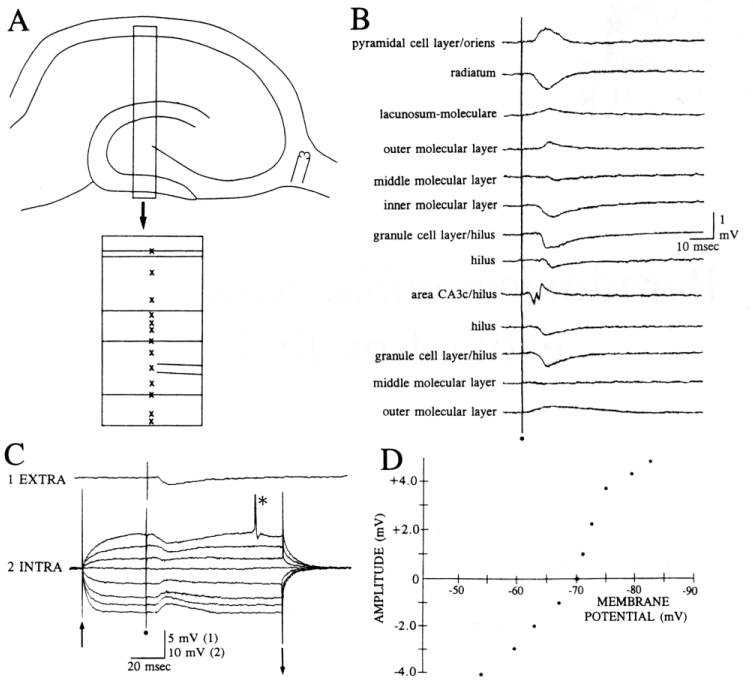

Fig. 1.

Fimbria stimulation evokes a negativity in the granule cell layer by extracellular recording that corresponds to a hyperpolarization of granule cells. A: a diagram of the slice shows where the stimulating electrode was placed in the fimbria and the area where recordings shown in B were made is boxed. The box is enlarged below, showing specific sites (marked by x's) for extracellular recording. B: responses to stimulation of the fimbria are shown for several sites in the slice, as illustrated in A. The same fimbria stimulus was used to elicit all responses. Stimulation occurred at the dot. C: simultaneous intracellular and extracellular recording of granule cell responses to fimbria stimulation. 1. Extra: extracellular recording from the granule cell layer. The recording site was close to the cell shown in part C2. 2. Intra: intracellular recordings from a granule cell. Eight superimposed responses of a granule cell to the same fimbria stimulus are shown. Intracellular responses were recorded during depolarizing or hyperpolarizing current steps except for the central trace, which was evoked without a current step. The start and end of the steps are marked by arrows. The action potential at the asterisk is truncated. Stimulation occurred at the dot. Membrane potential, −72 mV; resting potential, −79 mV. D: amplitudes of granule cell responses to fimbria stimulation are plotted as a function of membrane potential for the cell shown in C.

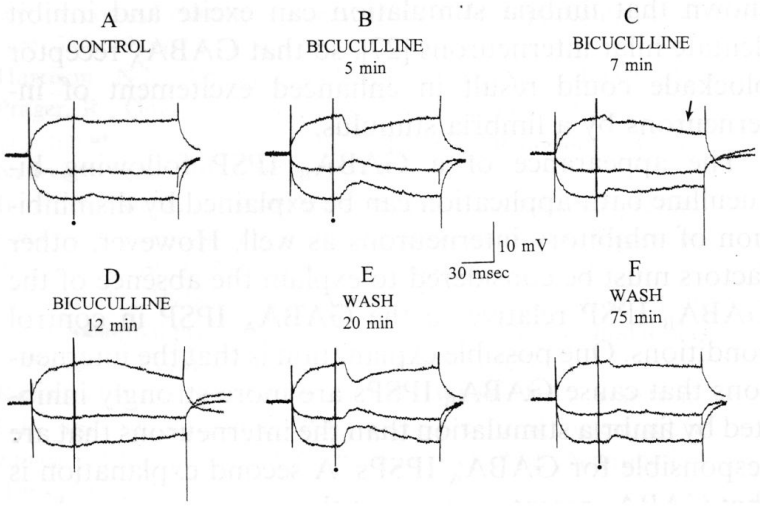

Fig. 2.

Bicuculline increased and then blocked the hyperpolarization evoked by fimbria stimulation. A: 3 responses to fimbria stimulation, triggered during hyperpolarizing or depolarizing current steps, are shown superimposed. Stimulation occurred at the dot. Membrane potential, −66 mV; resting potential, −82 mV. B: responses to the same fimbria stimulus are shown 5 min after 25 μM bicuculline was added to the buffer. Note the increase in IPSP amplitude. C: 7 min after bicuculline was added to the buffer, the IPSP was reduced and a second, later hyperpolarization was evident (arrow). D: at 12 min, the short latency IPSP was blocked, but the second hyperpolarization was not. E: 20 min after perfusion with drug-free buffer was resumed, stimulation produced a very large early IPSP but no late hyperpolarization. F: following prolonged perfusion with drug-free buffer, the response to fimbria stimulation was similar to control conditions.

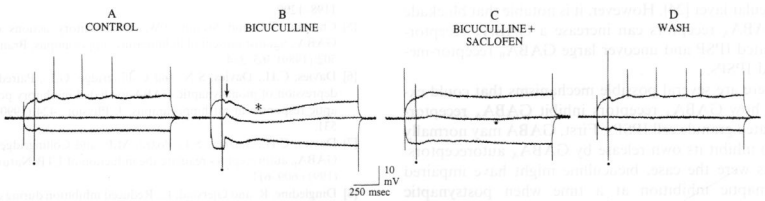

Fig. 3.

The fimbria-evoked late hyperpolarization, revealed after bicuculline application, was blocked by the $GABA_B$ receptor antagonist 2-hydroxy saclofen. A–D: 3 responses to an identical fimbria stimulus are shown superimposed, triggered during current steps (top and bottom traces) or without any current injection (central trace). In control (A), a small IPSP was recorded. Following reduction of the $GABA_A$ IPSP (B), a late hyperpolarization was evoked after the residual $GABA_A$ IPSP. The residual $GABA_A$ IPSP is marked by an arrow, the late hyperpolarization is marked by an asterisk. The late hyperpolarization was blocked by 500 μM 2-hydroxy saclofen (C) reversibly (D). Stimulation occurred at the dot. Action potentials are truncated. Membrane potential, −71 mV; resting potential, −85 mV.