Heterosynaptic Transformation of GABAergic Gating in the Hippocampus and Effects of Carbonic Anhydrase Inhibition

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ABSTRACT

Recordings from CA1 pyramidal cells were made in rat hippocampal slices (in vitro). Activation of cholinergic receptors associated with tetanization of GABAergic inputs from stratum pyramidale transformed the hyperpolarizing GABA-mediated inhibitory postsynaptic potentials into depolarizing responses of rat hippocampal CA1 pyramidal neurons. The synaptic transformation was characterized by a significant shift of reversal potential of postsynaptic responses toward positive membrane potentials. This effect lasted more than 1 h and changed the function of the GABAergic synapses from excitation filter to amplifier. This long-term synaptic transformation was prevented by carbonic anhydrase inhibitors or the presence of HEPES buffer, indicating a dependence on HCO₃⁻. The presence or absence of an associated activation of cholinergic with GABAergic inputs thus gates the information processing through the pyramidal cells and network, forming an amplified "center" of attention and a filtered "surround". Information flow through the neural circuit is thereby directed according to temporal association of the relevant signals.

Memory pharmacology that reverses memory decline or blocks the effects of past traumatic experience through the use of pharmacological agents has not yet been well characterized. The efficacy of memory therapeutics depends on our understanding of the basic mechanisms that characterize memory itself. Memories are thought to be due to lasting synaptic modifications in the brain. Synaptic modifications within memory traces have been linked to a number of mechanisms that involve multiple and interacting afferent pathways, neurotransmitters, messenger molecules, and gene products (Kornhauser and Greenberg, 1997; Alkon et al., 1998; Paulsen and Moser, 1998; Xiang et al., 1998). Some studies have correlated synaptic modifications, or biophysical and biochemical correlates, with behavioral learning and memory (Alkon et al., 1982, 1992; Bradford and McCabe, 1994; Xiang et al., 1998) and others identified long-lasting synaptic modifications such as long-term potentiation (LTP) or depression that are induced by electrophysiological stimulation (Christie et al., 1994; Teyler et al., 1995). Classical paradigms for induction of LTP or long-term depression typically involve tetanization or low-frequency stimulation of a single glutamatergic pathway, respectively. Memory impairments, such as in Alzheimer's disease, are, however, generally characterized by multiple deficits of neurotransmitters in the brain.

Targeting transmitter synapses in memory-related structures is among the most attractive ways to directly affect reception of relevant signals and how they are processed and stored as memory traces. The importance of cholinergic and GABAergic systems in hippocampus-dependent memory has been well established (Winkler et al., 1995; Paulsen and Moser, 1998). Acetylcholine (ACh) is crucial to attention, learning, and memory (Bartus et al., 1982; Buccafusco et al., 1995; Ohno et al., 1997; Robbins et al., 1997), and the generation of hippocampal θ rhythmic activity. Activation of the septal cholinergic inputs, a major cholinergic pathway to the hippocampus (Cooper and Sofroniew, 1996; Kalman et al., 1997), is thought to accompany associative learning (Day et al., 1991; Inglis et al., 1994; Inglis and Fibiger, 1995). Its disruption blocks spatial memory (Winson, 1978; Winkler et al., 1995). One GABAergic interneuron innervates a population of some 1000 pyramidal cells. These interneurons exert significant control on hippocampal network activity and synchronize the firing of pyramidal cells (Buhl et al., 1995; Cobb et al., 1995). However, functional interactions between cholinergic receptor activation and GABA synaptic modifications have remained somewhat obscure. Here, we investigate the physiological conditions underlying synaptic modification and induction of long-term synaptic transformation (LTT) of GABAergic synapses by cholinergic receptor activation. LTT has previously been reported to be induced by associating postsynaptic depolarization with s. pyr tetanization (Collin et
al., 1995) or intracellular administration of calexcitin (Sun et al., 1999), a memory signal protein (Alkon et al., 1998). It appears to depend on intracellular Ca\textsuperscript{2+} release, probably from the ryanodine receptors (for review, see Alkon et al., 1998). In the present study, we found that associated activation of heterosynaptic inputs without postsynaptic depolarization transforms GABAergic inhibition to excitation. Furthermore, the GABAergic synaptic transformation effectively switched an excitatory input filter to an excitatory input amplifier, altering direction of signal transmission through the network. The synaptic transformation and the transformed response were blocked or eliminated by acetazolamide and bicuculline (BIC), respectively, suggesting an involvement of HCO\textsubscript{3}⁻ flux through the chloride channels.

Materials and Methods

Brain Slices. Male Sprague-Dawley rats (125–150 g) were anesthetized with diethyl ether and decapitated. The hippocampal formation was removed and sliced (400 µm) with a McIlwain tissue chopper (Collin et al., 1995; Sun et al., 1999). Slices were maintained in an interface chamber (Medical Systems Corp., Greenvale, NY) at 32°C with continuous perfusion of artificial cerebrospinal fluid (aCSF). aCSF consisted of 125 mM NaCl, 3 mM KCl, 1.3 mM MgSO\textsubscript{4}, 2.4 mM CaCl\textsubscript{2}, 26 mM NaCHO\textsubscript{3}, 1.25 mM NaH\textsubscript{2}PO\textsubscript{4}, and 10 mM C\textsubscript{6}H\textsubscript{12}O\textsubscript{6}.

Electrophysiology. Intracellular recordings were obtained from CA1 pyramidal neurons using glass micropipette electrodes filled with 2 M potassium acetate or 0.1 M potassium methylsulfate, with measured tip resistance in the range 70 to 200 MΩ. Cells that show obvious accommodation (e.g., as shown in Figs. 1e, and 2, e and f), a key characteristic of pyramidal cells, were used in the study. Labeling the recorded cells exhibiting the characteristic with dye has previously revealed that the recorded cells are indeed pyramidal cells (Sun et al., 1999). Signals were amplified, digitized, and stored using AxoClamp-2B amplifier and DigiData 1200 with the P-clamp data acquisition and analysis software (Axon Instruments, Foster City, CA). S. pyr, stratum radiatum, and stratum oriens (s. oriens) were stimulated (about 200 µm from the recording electrode; Fig. 1a) using bipolar electrodes constructed of Teflon-insulated platinum iridium wire (25 µm in diameter, the approximate thickness of s. pyr; FHC Inc., Bowdoinham, ME). Monophasic hyperpolarizing PSPs were elicited by orthodromic single-pulse stimulation of interneuron.
rons in s. pyr (Collin et al., 1995). In some experiments, a stimulating electrode (about 400 μm from the other stimulating electrodes when two stimulating electrodes were placed) was also placed in s. oriens to activate cholinergic terminals and evoke ACh release (Cole and Nicoll, 1984), or in stratum radiatum to evoke gluatamatergic PSPs. Tetanization of s. pyr consisted of 10 trains, 10 pulses at control intensity (30–60 μA and 50 μs), 100 Hz, and a 0.5-s intertrain interval. Stimulation of s. oriens consisted of single pulses (20–60 μA and 50 μs), delivered at 1 Hz for 30 s.

**Drugs and Ligands.** Benzolamide (gift from T. H. Maren, University of Florida, Gainesville, FL) was applied into recorded cells (0.1 mM; 0.5 nA, 500 ms at 50% on cycles for 10 min through the recording electrode), Carbachol (CCH), physostigmine (PHY), BIC, acetazolamide, kynurenic acid, atropine, 6-cyano-7-nitroquinoxaline-2,3-dione, and D-(-)-2-amino-5-phosphonopentanoic acid (AP5) were from Sigma (St. Louis, MO) and were solubilized in aCSF in the noted concentrations and delivered to the slice chamber from an external reservoir.

**Results**

A single-pulse stimulus delivered to s. pyr elicited a hyperpolarizing IPSP (Fig. 1, b–d), resulting, mainly if not exclusively, from activation of the GABAergic inputs from the Basket interneurons (Bas) whose cell bodies and axons are restricted to s. pyr. The response magnitude depended on intensities of stimulation (Fig. 1, b–d), without revealing any obvious depolarizing excitatory postsynaptic potential (EPSP) components within the stimulation intensities used. The monophasic nature of the elicited PSPs was further indicated by evoking the PSPs over a range of membrane potentials, hyperpolarized or depolarized relative to the resting potential in eight neurons. Figure 1e illustrates the PSPs elicited by single-pulse stimulation of s. pyr over a range of membrane potential steps. The PSP reversed at a membrane potential of −78 mV (Fig. 1g), exhibiting a linear relationship between the membrane potentials and the evoked PSPs. The time to peak of the evoked response depended on the direction of the anion flux, with the maximum response at hyperpolarized membrane potentials (chloride efflux), occurring some 20 ms earlier than that evoked at resting membrane potentials (chloride influx; Fig. 1f). No detectable minor PSP components that exhibit a different reversal potential were observed. Bath application of 500 μM kynurenate (20 min), a broad-spectrum competitive antagonist for both aspartate (NMDA) and non-NMDA receptors (Collingridge and Lester, 1989; Sun, 1996), effectively abolished EPSPs of CA1 pyramidal cells evoked by stimulation of the Schaffer collateral pathway (Sch; by 95.8 ± 3.2%, n = 8, p < 0.05; Fig. 1h). This concentration of kynurenate produced no significant changes (−8.5 ± 0.7 mV prekynurenate versus −8.5 ± 0.8 mV during the application; n = 8, p > 0.05) in the IPSPs evoked by single-pulse s. pyr stimulation (Fig. 1i). Thus, these kynurenate results suggest that the single-pulse s. pyr stimulation did not evoke a significant glutamatergic EPSP component. The IPSPs, however, were blocked by the GABA<sub>δ</sub> receptor antagonist BIC (by >95%, n = 8, p < 0.05; 1 μM, 30-min perfusion; Fig. 2a), indicating the involvement of GABA<sub>δ</sub> receptors.

Single-pulse stimulation of s. oriens (1 Hz, 30 s) coincident with tetanization of s. pyr consistently induced LTT (Fig. 2, b and d, 7.1 ± 1.3 versus −7.4 ± 1.2 mV before the associated stimulation, n = 9, p < 0.05). Tetanization of s. pyr was applied near the end of s. oriens stimulation (i.e., coincident with pulses 25–30 of the 30-s s. oriens stimulation). This LTT does not appear to result from a simple blockade of a receptor-channel complex. Rather, it was associated with a shift (Fig. 2, e, f, and g) of the relationship between Bas-CA1 PSPs and membrane potential to the right, and a shift of the reversal potential to more positive potentials (from −78.5 ± 1.1 to −61.4 ± 1.2 mV, n = 9, p < 0.05). No obvious depolarization of the resting membrane potential was observed during and after the associative stimulation. The synaptic transformation induced by s. oriens stimulation-s. pyr tetanization was prevented by atropine (20 μM, n = 6; Fig. 2d), which did not affect IPSPs elicited by single-pulse stimulation of s. pyr. Tetanization of s. pyr alone, however, did not induce LTT, but significantly increased the evoked IPSPs (Fig. 2, c and d; from −7.6 ± 1.2 to −10.7 ± 1.0 mV, n = 5, p < 0.05). Single-pulse stimulation of s. oriens alone for 30 s before tetanization of s. pyr at the same intensity and frequency) effective in inducing the synaptic transformation in six cells tested (data not shown).

The involvement of ACh in the synaptic transformation was further examined by preceding s. pyr tetanization with a 20-min period of extracellular perfusion of CCH (10 μM), an ACh receptor agonist. Perfusion with 10 μM CCH alone for 20 min in the interface chamber did not depolarize the membrane resting potential (n = 18), consistent with the observation (Muller et al., 1988) that the occurrence of carbachol-induced depolarization of hippocampal neurons depends on the rate at which carbachol concentration is elevated in the tissue. Nor were epileptiform-like activities ever observed in the experimental period. Depolarization was consistently induced when using higher CCH concentrations (>100 μM), submission-type chamber, more rapid perfusion, or longer perfusion in brain slices (Muller et al., 1988). The CCH-s. pyr tetanization paradigm, however, transformed the IPSPs (−7.1 ± 0.6 mV) to depolarizing EPSPs (6.9 ± 0.9 mV, n = 9, p < 0.05; Fig. 3) and shifted the reversal membrane potential from −78.2 mV (n = 9) on average to more positive potentials (−56 mV on average; n = 9) for >1 h. We also tested the possibility that sufficient ACh is spontaneously released from an endogenous source(s) coincident with tetanization of s. pyr to induce the synaptic transformation. Consistent with this possibility, 20 min after PHY (an anticholinesterase, 20 μM), tetanization of s. pyr (without CCH) repeatedly induced a lasting synaptic transformation (Fig. 3; −6.9 ± 0.4 versus 2.4 ± 0.4 mV, n = 5, p < 0.05; Fig. 3g).

In the presence of CNQX (100 μM) and AP5 (50 μM), the synapse was not transformed by the CCH-s. pyr tetanization. To the contrary, the CCH-s. pyr tetanization paradigm consistently and significantly potentiated the IPSP amplitude (by 40 ± 8.8%, n = 4, p < 0.05) in the presence of CNQX and AP5. This latter result is consistent with the observation that s. pyr tetanization without postsynaptic depolarization potentiated IPSPs (Collin et al., 1995). Blocking the NMDA receptor subtype with AP5 alone was effective in preventing the long-term synaptic transformation (by 90.1 ± 8.5%, n = 5, p < 0.05). Blocking the non-NMDA receptor subtype did not, however, appear to be sufficient to prevent the synaptic transformation. The CCH-s. pyr tetanization consistently induced the transformation in the presence of CNQX alone (−7.0 ± 0.39 versus 8.3 ± 0.46 mV, n = 3, p < 0.05). How-
ever, once the transformation was induced, it was not changed by CNQX (100 \mu M) and AP5 (50 \mu M) (n = 4; Fig. 3c). These results suggest that although induction of the synaptic transformation requires activation of NMDA receptors or a network circuit involving activation of NMDA receptors, maintenance of the transformation does not. BIC (a GABAA receptor antagonist, 1.0 \mu M), however, eliminated the transformed PSPs (Fig. 3f; n = 6). Elimination of the depolarizing PSPs after the synaptic transformation by BIC suggests that the underlying current largely involves activation of GABAA receptor-activated channels.

In the presence of acetazolamide (1 \mu M, 30 min), a blocker of carbonic anhydrase but not the synthesis of HCO_3^-(Staley et al., 1995), the Bas-CA1 IPSPs did not undergo transformation. Thus, these IPSPs were not altered by single-pulse s. oriens-s. pyr tetanization (Fig. 4a; 98.9 ± 2.7\%, 30 min after compared with 100\% control value, n = 8, p > 0.05). When perfused externally, bicarbonate buffer also prevented induction of the synaptic transformation (Fig. 4c; 98.5 ± 3.2\%, 30 min after single-pulse s. oriens-s. pyr tetanization compared with 100\% control value, n = 7, p > 0.05). Since carbonic anhydrase was previously shown to exist in the pyramidal cells (Pasternack et al., 1993), we injected the membrane-impermeant carbonic anhydrase inhibitor benzo-}

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**Fig. 2.** Associating single-pulse stimulation of s. oriens with pyramidal tetanization induced long-term synaptic transformation. Single-pulse stimulation of s. pyr (50 \mu A, 50 \mu s) evokes a BIC-sensitive IPSP (a). Postsynaptic responses of a pyramidal cell (b) to single-pulse stimulation of s. pyr before (control) and 60 min after s. pyr tetanization (s. oriens-s. pyr Tet; 10 trains of 50 \mu A, 50 \mu s, 10 pulses/train at 100 Hz, 0.5-s intertrain interval), associated with single-pulse s. oriens stimulation (50 \mu A, 50 \mu s) (at the arrow). Tetanization of s. pyr alone (10 trains of 50 \mu A, 50 \mu s, 10 pulses/train at 100 Hz, 0.5-s intertrain interval) does not induce long-term transformation, but potentiates IPSPs (c), as shown with representative IPSPs in response to single-pulse stimulation (50 \mu A, 50 \mu s) of s. pyr before (control) and 60 min (s. pyr Tet) after s. pyr tetanization. Group data show that long-term synaptic transformation is induced by stimulating cholinergic pathway in s. oriens and tetanization of s. pyr (s. oriens-s. pyr Tet) but is sensitive to atropine (atropine + s. oriens-s. pyr Tet), whereas s. pyr tetanization alone is not effective (d; as means ± S.E.M.). Associative tetanization of s. pyr with s. oriens single-pulse stimulation (f) shifts the relationship between the evoked Bas-CA1 PSP and membrane potentials to the right (h), compared with that of the control (e).
tion with the tetanization amplified excitatory Sch inputs (Fig. 4g). Thus, weak signals are amplified in the cells with the synaptic transformation, whereas strong excitatory signals cannot successfully pass through the network under the enhanced Bas inhibition.

Discussion

The present study shows, for the first time, that the hyperpolarizing IPSPs of rat hippocampal field CA1 pyramidal neurons in response to s. pyr stimulation undergo a reversal in polarity (LTT) after the temporally associated activation of cholinergic and GABAergic receptors. The associated activation of multisynaptic inputs is at least as effective in LTT induction as the postsynaptic depolarization-s. pyr tetanization paradigm reported previously (Collin et al., 1995). Temporal association of different inputs thus may code neural information, in line with the view that recognition memory is not mediated by a single neurotransmitter type (Steckler et al., 1998). The synaptic reversal was sensitive to carbonic anhydrase inhibitors. The critical role of cholinergic receptor activation on the synaptic transformation was further demonstrated by the effectiveness of atropine and the anticholinesterase PHY. The results are consistent with an in vivo report that carbonic anhydrase inhibition reduces the \( \theta \) rhythm (Sone et al., 1998).

The observed synaptic transformation does not appear to involve a masked excitatory component, such as glutamatergic EPSPs. Microstimulation was delivered to the area remote to major excitatory terminal inputs and the stimulation at the selected intensity did not directly activate the pyramidal cells. Thus, the evoked IPSPs are monophasic and showed little or no change in magnitude with an effective blockade of the glutamatergic receptors. The lack of a depolarizing component of the IPSP was further confirmed by a single reversal potential for s. pyr-elicited responses. Furthermore, the evoked IPSPs and transformed depolarizing PSPs were abolished by blocking the GABAA receptors, whereas an effective blockade of the glutamatergic receptors was ineffective after the synaptic transformation.

However, induction of the synaptic transformation was prevented by pretreatment with the glutamatergic receptor antagonists AP5 and CNQX, AP5, or kynurenic acid, but not with CNQX alone. This indicates that the excitatory amino acid L-glutamate is released in the in vitro preparation and its activation on the NMDA receptors is required for induction of the synaptic transformation. Whether the glutamate release is a result of s. pyr tetanization or occurs spontaneously is unknown at this time. It is probable that some spontaneous glutamatergic activity is present in vivo and sufficient for synaptic transformation to occur. Disinhibition of inhibitory inputs might also result in enhanced activity of the principal cells. Another possibility is that the induction

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**Fig. 3.** Carbachol and physoestigmine mimic s. oriens stimulation in inducing long-term synaptic transformation. Single-pulse s. pyr stimulation (50 \( \mu \)A, 50 \( \mu \)s at arrowhead) evoked a monophasic IPSP (a) in the hippocampal CA1 pyramidal neuron. Long-term synaptic transformation is induced by simultaneous CCH perfusion and s. pyr tetanization (b). After induction by the CCH-s. pyr tetanization paradigm, the synaptic transformation persists with CNQX (100 \( \mu \)M) and APV (50 \( \mu \)M) perfusion for 30 min (c). In a different cell, single-pulse s. pyr stimulation (50 \( \mu \)A, 50 \( \mu \)s at arrowhead) evoked a monophasic IPSP (d). Long-term synaptic transformation is induced with simultaneous CCH perfusion (10 \( \mu \)M, 20 min) and s. pyr tetanization (e). After induction by the CCH-s. pyr tetanization paradigm, the synaptic response is eliminated with BIC (1 \( \mu \)M) perfusion for 20 min (f). Group data (g) show that long-term synaptic transformation (as means \( \pm \) S.E.M.) induced by a 20-min perfusion with either CCH or PHY and tetanization of s. pyr (10 trains, 10 pulses at 100 Hz, 0.5-s intertrain interval).
may involve activation of a network circuit, including activation of NMDA receptors although its maintenance does not. Response of the recorded cells to activation of a network circuit, if evoked, however, would be delayed and not jeopardize the immediate synaptic response analyzed in the study. Thus, the synaptic transformation induced in our study differs from an effect reported by Kaila et al. (1997) and Taira et al. (1997). In their studies, tetanic Sch stimulation, instead of the s. pyr tetanization/cholinergic activation in the present study, elicits a lasting GABAergic depolarizing PSP.

Acetazolamide, an inhibitor of carbonic anhydrase, was shown to reduce or eliminate flux of HCO$_3^-$ in hippocampal pyramidal neurons underlying a depolarizing PSP (Staley et al., 1995). Acetazolamide also prevents the synaptic transformation. Activity of carbonic anhydrase in the CA1 pyramidal cells is essential since intracellular application of benzolamide, a membrane-impermeant carbonic anhydrase inhibitor, effectively blocked the synaptic transformation. The results of the present study are consistent with an induction of a depolarizing transmembrane HCO$_3^-$ flux that underlies the synaptic transformation. The relatively brief time course of the transformed PSPs, compared with the time course of IPSPs before the synaptic transformation, suggests that the HCO$_3^-$ flux may be limited by its availability. It is possible, then, that during a transformed PSP, the GABA-activated channel(s) becomes more rapidly inactivated. Indeed, the channel appears to operate with different characteristics when conducting an efflux or influx of anion, perhaps reflecting a flux direction-dependent property.

GABAergic postsynaptic depolarizing responses are observed in neonatal brains (Leinekugel et al., 1999) and could be induced by prolonged activation of GABA$_A$ receptors (Staley and Proctor, 1999) or the use of neuroactive steroids (Burg et al., 1998) in the adults. The present results demonstrate a persistent heterosynaptic modification induced by synergy of neurotransmitters. Synergy between cholinergic, GABAergic, and glutamatergic activation is suggested for the synaptic transformation, consistent with the observation that in hippocampal field CA1, application of GABA in association with Sch tetanization also produces a transient depolarizing response (Wong and Watkins, 1982). The transformed synaptic inputs from the Bas interneurons are also shown to provide a mechanism to direct or gate signal flow through the hippocampal network. These interneurons innervate the perisomatic region of the pyramidal cells. Thus, bursting activity from the interneurons in the absence of associated cholinergic activity enhances the inhibitory control of the pyramidal cells, powerfully blocking excitatory signal transfer through the hippocampal circuit. With the temporal association of cholinergic activity, however, the same type of GABAergic activity amplifies excitatory signal strength. The differentiation in responses according to the nature and temporal association of relevant signals enables the network to perform signal processing and gate information flow and direction. The occurrence of the switch is controlled postsynaptically. The formation of a functional “center” of attention (those transformed) and a “surround” (those enhanced) would dramatically increase the signal-to-noise ratio. The in vitro synaptic changes analyzed here are consistent with in vivo studies that implicate ACh in arousal, attention, and...
memory mechanisms (Dickinson-Anson et al., 1998; Perry et al., 1999; Sun et al., 2001). These results suggest, therefore, that attention and arousal (mediated by ACh) and signaling (via the hippocampal trisynaptic circuit from the entorhinal cortex through the dentate gyrus and field CA3 to field CA1) could interact with GABAergic synaptic activation. This activation could, via transformation of IPSPs to EPSPs, selectively amplify synaptic weights relevant to a particular memory, forming the signal center of attention. In this switching cascade, the highly efficient carbonic anhydrase appears to play an important role. Consistent with the role of carbonic anhydrase in hippocampus-dependent memory are observations that administration of a carbonic anhydrase inhibitor in vivo reduces hippocampal theta activity (Sone et al., 1998), which is believed by many to gate or facilitate memory information processing in the hippocampus, and impairs rat spatial watermaze performance (Sun et al., 2001). Agents that inhibit carbonic anhydrase may have clinical value for temporary suppression of traumatic memories, such as in surgery or post-traumatic stress disorder.

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References


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