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Presynaptic Inhibition of GABA_A Receptor-Mediated Unitary IPSPs by Cannabinoid Receptors at Synapses Between CCK-Positive Interneurons in Rat Hippocampus

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Ali AB. Presynaptic inhibition of GABA_A receptor-mediated unitary IPSPs by cannabinoid receptors at synapses between CCK-positive interneurons in rat hippocampus. *J Neurophysiol* 98: 861–869, 2007. First published June 13, 2007; doi:10.1152/jn.00156.2007. There is growing evidence to link cholecystokinin (CCK)-positive interneurons and anxiety disorders. Despite this, little is known about the physiology and pharmacology of synaptic interactions between CCK-positive interneurons. This study aims to investigate the local circuit connections among CCK-positive Schaffer collateral associated (SCA) interneurons in stratum radiatum (SR) and their modulatory interactions using paired whole cell recordings combined with biocytin and double immunofluorescence labeling in slices of rat hippocampus. The cell bodies of SCA interneurons were located in SR, and their sparsely spiny dendrites projected toward s. pyramidale (SP) and along SR. Their axons innervated SR, SP, and s. oriens (SO) with predominant ramification in SR. These cells were immunopositive for CCK and immunonegative for parvalbumin (PV). SCA interneurons often displayed an accommodating firing pattern with or without a “sag” in response to hyperpolarizing current injection. Pairs of these cells exhibited electrical coupling and reciprocal chemical connections in which inhibitory postsynaptic potentials (IPSPs) displayed powerful frequency-dependent facilitation and augmentation. The synaptic connections were modulated by the endogenous cannabinoid receptor (CB) agonist, anandamide and by depolarization-induced suppression of inhibition (DSI), both of which reduced the amplitude of unitary IPSPs to 50% of control and increased the number of apparent failures of transmission. These effects were blocked by the CB1 receptor antagonist, AM-251. I suggest that synaptic facilitation between CCK-positive SCA interneurons may modify the onset of CB1 receptor-mediated regulation of inhibition, thereby affecting spike timing, and that this process could influence the expression of anxiety.

INTRODUCTION

CCK-positive GABAergic interneurons are thought to act as modulators that adapt network activity to behavioral states (Freund 2003). There are two populations of CCK immunoreactive interneurons in the CA1 region of the hippocampus; CCK-positive basket cells, which target proximal regions of postsynaptic neurons (Freund et al. 1986; Kawaguchi and Kubota 1997) and CCK-positive Schaffer collateral associated (SCA) interneurons, which target distal dendrites and dendritic spines of postsynaptic neurons (Cope et al. 2002; Pawelzik et al. 2002; Vida et al. 1998). CCK-positive interneurons also express a high level of cannabinoid type 1 (CB1) receptors (Marsicano and Lutz 1999; Tsou et al. 1999), located on their

GABAergic terminals (Hajos et al. 2000; Katona et al. 1999, 2000). These receptors regulating rhythmic activity of the network (Hajos et al. 2000) mediate depolarization-induced suppression of inhibition (DSI) (Kreitzer and Regehr 2001; Ohno-Shosaku et al. 2001; Trettel and Levine 2003; Wilson and Nicoll 2001) and are thought to be responsible for behavioral effects of cannabinoids, including the impairment of hippocampal memory formation (Hampson and Deadwyler 1998).

DSI is the reduction of GABA_A receptor-mediated inhibition in response to depolarization of the postsynaptic neuron. DSI requires the opening of voltage-dependent calcium channels in the postsynaptic cell and is thought to involve a retrograde signal that acts presynaptically on inhibitory terminals (Alger et al. 1996; Morishita and Alger 1997).

Previous studies in the hippocampus and neocortex have shown that the axon of an individual single pyramidal cell can exhibit several distinct activity-dependent patterns of neurotransmitter release that correlate with the types of postsynaptic target neuron (Ali and Nelson 2006; Ali and Thomson 1998; Ali et al. 1998, 2007; Beierlein and Connors 2003; Reyes et al. 1998). In CA1, basket and bistratified cells receive excitatory postsynaptic potentials (EPSPs) that display paired-pulse and brief train depression (Ali et al. 1998), and in striking contrast, EPSPs elicited in oriens lacunosum molecular (OLM) cells display paired pulse and brief train facilitation (Ali and Thomson 1998). It is these excitatory properties and the patterns of presynaptic activity that influence the behavior of specific classes of interneurons during specific network oscillations; however, these properties cannot alone explain why the same classes of interneurons behave differently during different network oscillations (Klausberger et al. 2003; Somogyi and Klausberger 2005). For example, the OLM cells are active and fire rhythmically at the trough of theta waves when pyramidal cells are active but remain silent during sharp waves in vivo (Klausberger et al. 2003). This suggests that other factors determine their firing properties and in turn modulate their postsynaptic targets in a brain-state-dependent manner, perhaps the influence of other interneurons? To assess factors that determine the firing properties of interneurons, I have examined the physiology and pharmacology of local circuit connections among CCK-positive interneurons in SR and their modulatory interactions using dual whole cell recordings combined

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with biocytin and double immunofluorescence labeling in hippocampal slices from P18- to 23-day-old rats.

METHODS

Slice preparation

Male Wistar rats (postnatal day, P18–23) were anesthetized by an intraperitoneal injection of sodium pentobarbitone (60 mg/kg Euthatal, Merial, UK) and perfused transcardially with 50–100 ml ice-cold modified artificial cerebrospinal fluid (ACSF). This modified ACSF contained (in mM) 248 sucrose, 25.5 NaHCO₃, 3.3 KCl, 1.2 KH₂PO₄, 1.0 MGSO₄, 2.5 CaCl₂, and 15 D-glucose, equilibrated with 95% O₂–5% CO₂. The animals were then decapitated and the brain removed; these procedures comply with British Home office regulations for the use of animals.

Coronal sections of cerebral cortex, 300–330 μm thick, were cut using a vibratome (Leica). The slices were incubated for 1 h in standard ACSF containing (in mM) 121 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 20 glucose, and 5 pyruvate and equilibrated with 95% O₂–5% CO₂. For recordings, slices were transferred to a submerged-style chamber and perfused at 1–2 ml/min with the standard ACSF.

Paired recordings

Simultaneous dual whole cell somatic recordings were made in current clamp between electrophysiologically identified CA1 SR interneurons. Cells of each recorded pair were visually selected using video-microscopy under near-infrared differential interference contrast (DIC) illumination. Interneurons were selected with round or oval somata and further characterized from their firing properties. Experiments were conducted at room temperature (20–22°C) with patch pipettes (resistance: 8–10 MΩ) pulled from borosilicate glass tubing and filled with an internal solution containing (in mM) 144 K-gluconate, 3 MgCl₂, 0.2 EGTA, 10 HEPES, 2 Na₂-ATP, 0.2 Na₂-GTP, and 0.02% wt/vol of biocytin (pH 7.2–7.4, 300 mosM).

Singles, pairs (with an interspike interval of 50 ms), or trains of presynaptic action potentials (APs) were elicited by injecting short (5–10 ms) pulses of depolarizing current into the presynaptic interneuron, repeated at 0.33 Hz (SEC 05L/H, npi electronics, GmbH). Electrical synapses between pairs of interneurons were studied by injecting 200-ms current pulses ranging from –0.1 to +0.1 nA in one cell and observing the passive transfer in the other.

Postsynaptic responses recorded in interneurons were amplified, low-pass filtered at 2 kHz, digitized at 5 kHz using a CED 1401 interface, and recorded on disc for off-line analysis.

DSI protocol

To investigate whether a release of retrograde messenger, in particular endocannabinoids could be activated from the postsynaptic SCA interneuron, a DSI protocol was designed, a schematic of this protocol is illustrated in Fig. 4*Ab*. The postsynaptic membrane potential was held at a fixed membrane potential of –55 mV for periods of 1.5 s repeated at 0.33 Hz. In control conditions (pre DSI), the presynaptic APs were elicited without any postsynaptic depolarization. However, to elicit DSI, the postsynaptic membrane was depolarized by injecting a 200-ms depolarizing step that elicited a train of three to six postsynaptic action potentials. Presynaptic APs were then elicited 900 ms after the end of the depolarizing step to allow the complete relaxation of the postsynaptic membrane currents. The duration of recording analyzed (pre and post DSI) was 90 s, which included 30 IPSPs.

Drugs

Pharmacology was performed on IPSPs elicited by single presynaptic action potentials that were >0.3 mV in average peak amplitude

(*n* = 9 pairs with pharmacology) for the effect of the drug and DSI to be seen easily.

Data were collected in the presence of bath-applied 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-2-amino-5-phosphonopentanoic acid (D-AP5, 50 μM), (RS)-α-methyl-4-carboxyphenylglycine (MCPG) (1 mM), (RS)-α-cyclopropyl-4-phosphonophenylglycine (CPPG) (100 μM), and CPG55845 (100 μM), naloxone (100 μM) to block AMPA/kainate, N-methyl-D-aspartate (NMDA), mGluR, GABA_B, and opioid receptors (all from Tocris, Bioscience).

The endogenous CB receptor agonist, anandamide (in water soluble emulsion; 14 μM) and CB1 receptor antagonists AM-251 (5 μM; Tocris) were used to study the CB1 receptor pharmacology of IPSPs elicited by SCA interneurons.

Data analysis

Data were acquired and analyzed using Signal software (Cambridge Electronic Design, Cambridge, UK) and in-house software. Individual sweeps were observed and either accepted, edited, or rejected according to the trigger points that would trigger measurements and averaging of the IPSPs during subsequent data analysis. Averaging of IPSPs was triggered from the rising phase of the presynaptic spike. The IPSP 10–90% rise times and width at half-amplitude were measured from averaged IPSPs. Single-sweep IPSP amplitudes were measured from the baseline to the peak of the IPSP. Average amplitudes are given as mean ± SD obtained from 150 to 500 sweeps. The electrophysiological characteristics of the recorded cells were measured from their voltage responses to 500-ms current pulses between –0.2 and +0.1 nA in amplitude. The IPSP rise times (10–90%) were measured from averaged IPSPs elicited by single APs. Apparent failures of synaptic transmission were counted assessed by eye and counted manually. Selection and averaging of these apparent failures resulted in no measurable postsynaptic response.

Coupling coefficients were calculated as the ratio between the change of voltage transfer in the noninjected cell and that of the current injected cell.

Statistical significance was analyzed by Student's paired *t*-test.

Morphology

Slices containing biocytin-filled cells were fixed overnight in 4% paraformaldehyde plus 0.2% saturated picric acid solution in 0.1 M phosphate buffer (PB), pH 7.2 at 4°C for immunofluorescence or in 1.25% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M PB for standard Avidin-HRP-DAB processing (see following text). Extensive rinses were carried out between each step using phosphate-buffered saline (PBS, 0.1 M). The sections were freeze-thawed over liquid nitrogen after cryoprotecting for 2 × 10 min in 10% sucrose, 2 × 20 min in 20% sucrose with 6% glycerol, and 2 × 30 min in 30% sucrose and 12% glycerol. The sections then followed either a double immunofluorescence (shorter recordings of 10–40 min) or a standard biocytin labeling protocol for longer recording (40–90 min) (for a detailed protocols, see Hughes et al. 2000).

Double immunofluorescence

After rapid freeze–thawing, the sections were washed in PB and then incubated in 1% sodium borohydride (NaBH₄) solution in 0.1 PB for 30 min. After further washes in PB to remove NaBH₄, slices were incubated in normal blocking serum diluted in 0.1 M PBS for 30 min. Both fluorescent-tagged secondary antibodies used were raised in goat, 10% normal goat serum (Sigma). The cells recorded in this study were incubated in antibodies to parvalbumin (PV) and CCK. These antibodies were raised against different antigenic targets, e.g., rabbit anti-PV (R301, source, Prof. Baimbridge, 1:1,000 dilutions) (Baimbridge and Miller 1982) and mouse anti-gastrin/CCK (MAB 9303, source, Cure, UCLA, 1:3,000 dilution). Primary antibody mixtures were made up with 3 mg

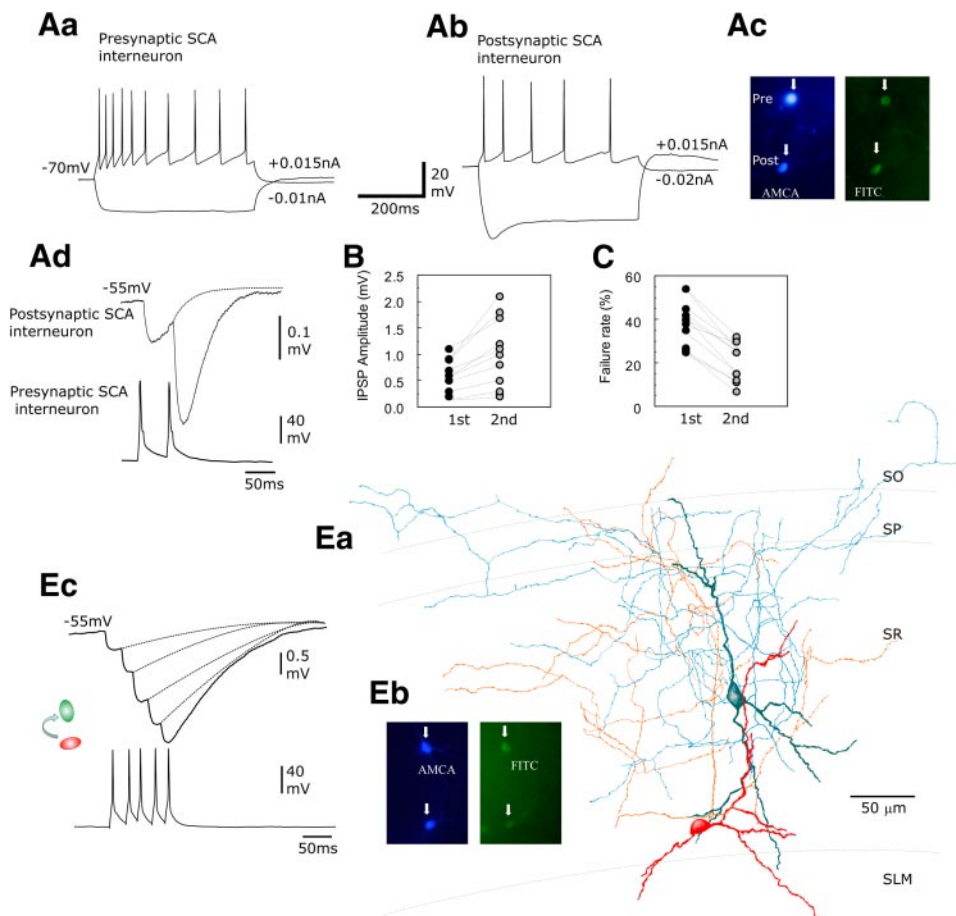


FIG. 1. Properties of Schaffer collateral associated (SCA) interneurons in stratum radiatum (SR). Interneurons in SR displayed an accommodating firing pattern without (*Aa*) or with (*Ab*) a “sag” in response to hyperpolarizing current injection. *Ac*: immunofluorescence of synaptically connected interneurons shown in *Aa* and *Ab* marked with AMCA to show biocytin-labeled cells and FITC to show that they were immunopositive for cholecystokinin (CCK). *Ad*: inhibitory postsynaptic potentials (IPSPs) elicited in SCA interneurons displayed synaptic facilitation. *B* and *C*: plot of 1st and 2nd IPSP amplitudes and failure rates for individual pairs. *Ea*: reconstruction of 2 CCK-labeled cells (*Eb*) and their synaptic connection (*Ec*), which displayed brief train facilitation.

bovin serum albumin (BSA; Sigma) per milliliter of diluents containing peroxidase-labeled ABC Elite (ABC-peroxidase; Vector Laboratories) made in PBS and incubated over night. The sections were washed in PBS prior to incubating in secondary antibodies and avidin macromolecules tagged with different fluorescent markers. This cocktail contained goat anti-rabbit IgG (diluted to 1:600, Molecular Probes) conjugated to Texas red, goat anti-mouse IgG (diluted 1:160; Sigma) conjugated to fluorescein isothiocyanate (FITC) and Avidin conjugated to 7-amino-4-methylcoumarin-3-acetic acid (AMCA) (1:250, Vector laboratories). After 3 h of incubation at room temperature, the sections were washed in PBS and mounted onto glass slides in 50% glycerol in PBS and cover slipped. The sections were then examined for fluorescent labeling using a Leica DMR microscope with appropriate filter blocks to visualize FITC, Texas red and AMCA respectively at $\times 40$ magnification (see Hughes et al. 2000; for further details). After immunohistochemical detections, the sections were processed to reveal the biocytin labeled cells as described in the following text.

Biocytin labeling

The sections were incubated overnight in Vector ABC-peroxidase (1:200) at 4°C . The peroxidase group was revealed using 3'3 diamino-benzidine as the chromogen (Vector DAB kit). The visualized cells were intensified with 0.1% osmium tetroxide, and the sections were cleared by dehydration in an ascending series of alcohols to 100%, and then embedded in Durcupan resin (Agar Scientific). The cells were reconstructed using a Zeiss Axioskop with attached camera lucida.

RESULTS

Within the SR interneurons were sparse and often only two to four pairs of neurons per slice could be tested to determine

whether they were synaptically or electrically connected. The probability of finding a connection between two SR interneurons was low. Of 40 experiments performed in the SR and SR/SLM border of CA1, 240 pairs of interneurons were tested: connections were found between 22 pairs of cells; 17 of these pairs were chemically connected (2 were reciprocal), 2 pairs were electrically coupled, and 3 pairs were both electrically and chemically coupled.

Anatomical properties of SCA interneurons

The interneurons selected for this study had multipolar round, oval, or pyramid-like cell bodies located in the SR. Their sparsely spiny dendrites projected predominantly in the SR, although some dendrites had a preferential horizontal projection, while others had a more vertical preferential, (see Figs. 1*Ea*, 2, *Aa* and *Ba*, and 3*A*). However, their axonal arbors were similar; a single axon originated from the cell body or a primary dendrite, innervated the SR, SP, and SO with predominant ramification in SR. These interneurons resembled SCA interneurons described previously (Cope et al. 2002; Pawelzik et al. 2002; Vida et al. 1998).

The pre- and postsynaptic SCA cells included in this study were all immuno-positive for CCK: two examples are shown in Fig. 1 (*Ac* and *Eb*). The cells were immuno-negative for PV.

Electrophysiological properties of SR interneurons

SR interneurons displayed an adapting firing pattern with ($n = 3$) or without ($n = 18$) a “sag” in response to hyperpo-

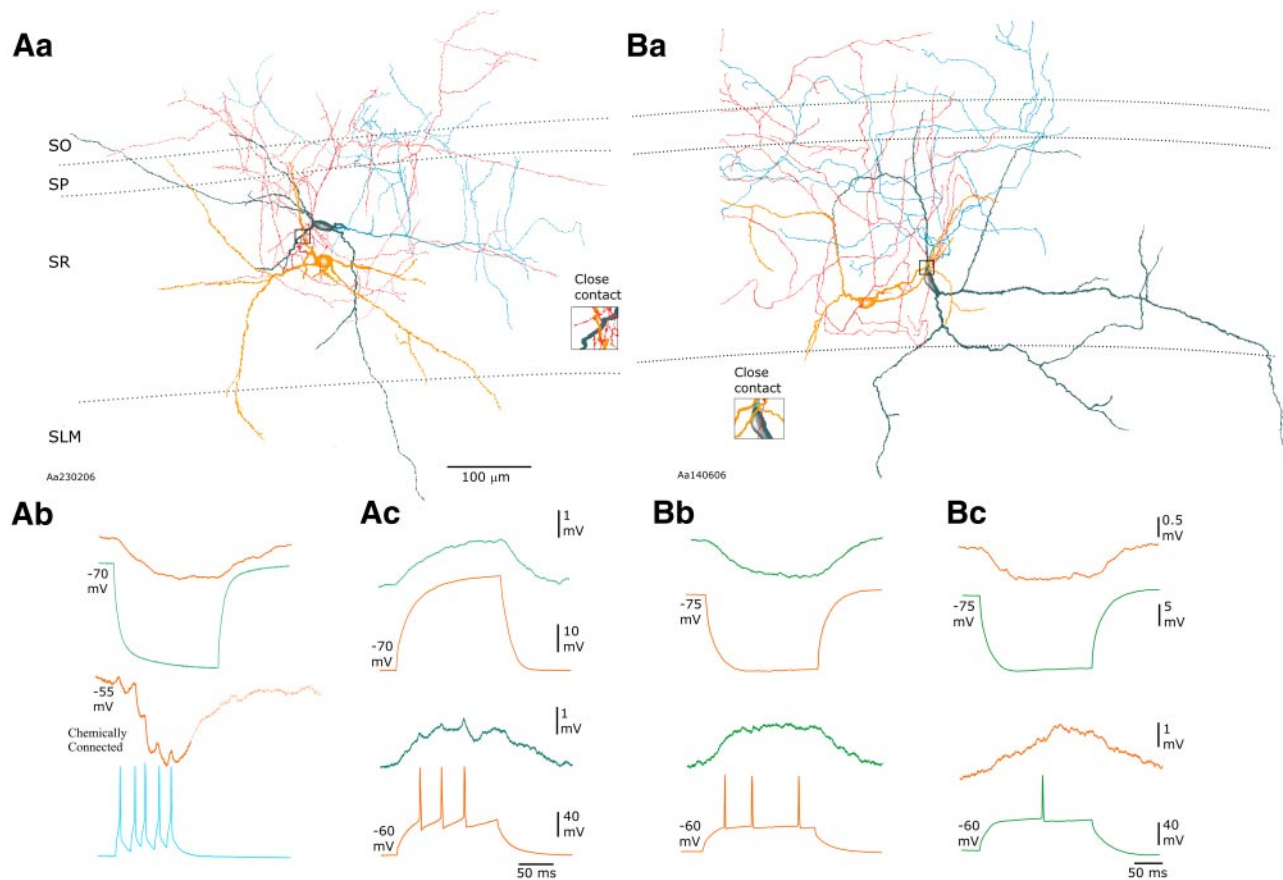


FIG. 2. Morphology and physiology of electrically coupled SCA interneurons in SR. *Aa* and *Ba*: reconstruction of electrically coupled SCA interneurons. The dendrites are shown in orange (red axon) and turquoise (blue axon), which spanned along SR and sometimes crossed into the dentate gyrus. The axons innervated SR, s. pyramidale (SP), and s. oreins (SO). Close contacts were made dendrodendritically (*Aa*) or dendrosomatically (*Ba*). *Ab* and *Ac*: pair of SCA interneurons connected both electrically and synaptically. Although the passive transfer of current is displayed in both directions, IPSPs recorded at -55 mV in response to a train of presynaptic action potentials were elicited in 1 direction only in this pair. *Bb* and *Bc*: passive transfer of hyperpolarizing and depolarizing current in both directions in the pair of SCA interneurons illustrated in *Ba*.

larizing current injection (Fig. 1, *Aa* and *Ab*). In each case, however, the action potential properties were similar ($n = 21$), and on average, spike widths at half-amplitude were 1.6 ± 0.33 ms. The spikes were followed by an afterhyperpolarization (AHP) of -12 ± 1 mV in amplitude and 5.7 ± 2.5 ms in duration (AHP width at half-amplitude). The spike amplitudes (measured from spike threshold to peak) were 66 ± 15 mV, and firing showed frequency adaptation and accommodation. For interneurons that did not respond with a sag to hyperpolarizing current injection, the input resistance measured from voltage response to a -0.05 nA current pulse was 300 ± 100 M Ω with a time constant of 24 ± 16 ms. For cells that displayed pronounced sag to the input resistance measured at the peak of the voltage response to a -0.1 nA hyperpolarizing current pulse delivered from a membrane potentials of -70 mV was 403 ± 36 M Ω , and at the end of the 500 ms current pulse, the input resistance was 310 ± 29 M Ω . The time constant for these cells was 29 ± 6 ms.

Electrical coupling of interneurons in the SR

Five of 22 pairs of SCA interneurons that yielded a connection were electrically coupled via dendrodendritic (Fig. 2*Aa*) or dendrosomatic (*Ab*) contacts. When SCA interneurons were electrically connected, a change in the voltage as a result of

current injection in one cell was passively transferred to the noncurrent injected cell, and this electrical coupling was always bidirectional. Figure 2 demonstrates the passive transfer of voltage from one interneuron to another. The example pair shown in Fig. 2*Ab* was also chemically connected. The electrical coupling was significantly stronger in one direction within a pair (paired *t*-test, $P < 0.05$, $n = 5$). The average mean coupling coefficient was $6 \pm 2.8\%$ ($n = 5$, range: 3–10%) in one direction and $4.4 \pm 2.9\%$ ($n = 5$, range: 2–9%) in the other direction. These values were similar for hyper- and depolarizing current injections.

Properties of IPSPs elicited in SCA interneurons

Across 20 pairs of synaptic connections between SCA interneurons, the average unitary IPSP peak amplitude after the first presynaptic action potential was 0.6 ± 0.41 mV. The 10–90% rise time and the width at half-amplitude were, on average, 7.0 ± 1.38 and 41.1 ± 12.5 ms, respectively. All connections between SCA interneurons displayed paired pulse and brief train facilitation and augmentation (Fig. 1, *Ad* and *Ec*). The average second IPSP elicited at an interspike interval of 50 ms, had a peak amplitude of 0.9 ± 0.2 mV (range, 0.3 and 2 mV), and was between two and three times the amplitude of the first IPSPs (paired *t*-test, $P < 0.05$, $n = 15$). Figure

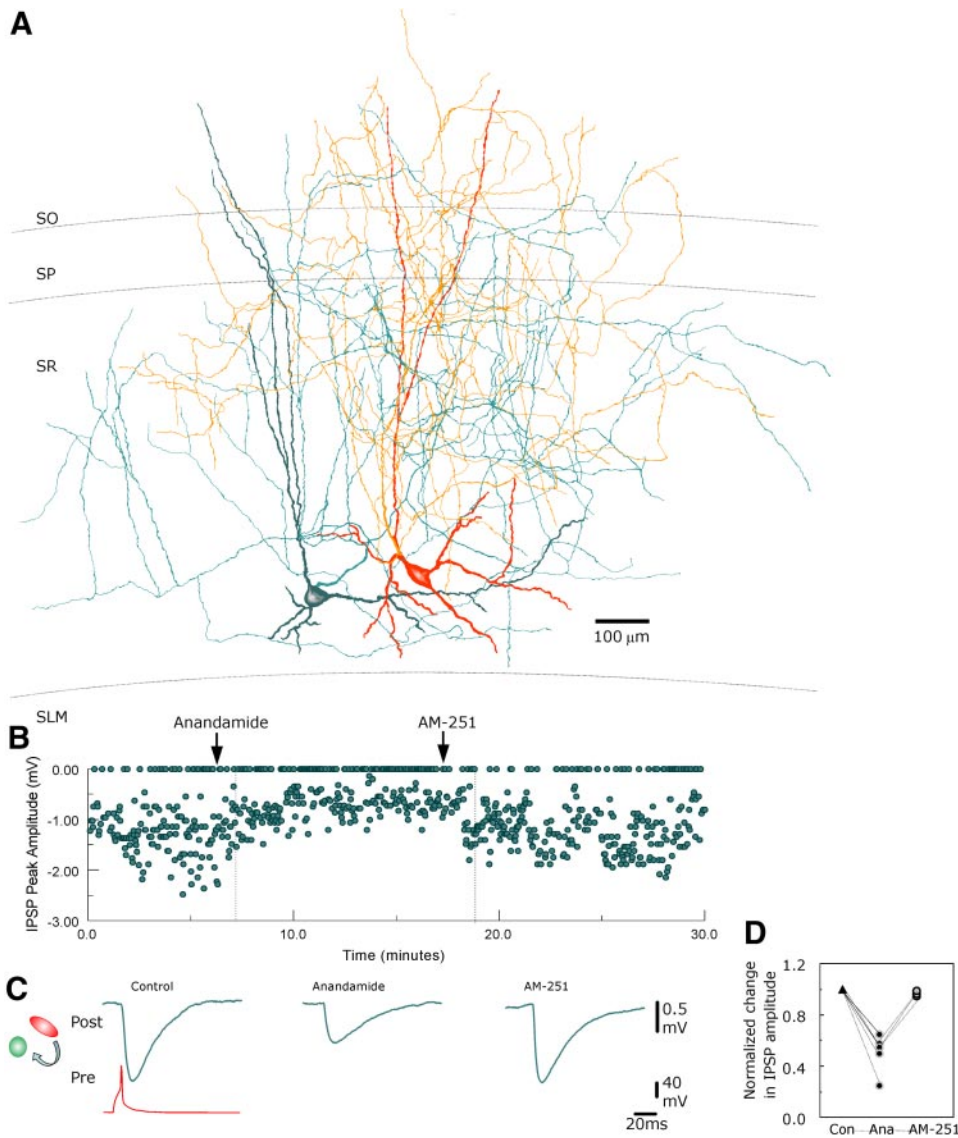


FIG. 3. SCA interneuron IPSPs are sensitive to CB1 receptor pharmacology. *A*: reconstruction of a synaptically connected pair of interneurons in SR. The dendrite of the presynaptic cell is shown in red and axon shown in orange and the dendrites of the postsynaptic cell is shown in dark turquoise and axon in light turquoise. The axon of both cells innervated mostly in SR with some branches in SP and SO. *B*: plot of the peak amplitude of unitary IPSPs during the time course an experiment in control condition, bath application of a CB receptor agonist, anandamide ($14 \mu\text{M}$) and subsequent addition of CB1 antagonist AM-251 ($5 \mu\text{M}$). *C*: average IPSPs in each condition; IPSPs decreased the peak amplitude with anandamide, which almost returned to control values after subsequent addition of the AM-251. *D*: change in IPSP average amplitude with anandamide and AM-251 in 6 pairs.

1, *B* and *C*, illustrates the first and second IPSP average amplitudes and failure rates for individual pairs. For all pairs, the coefficient of variation (CV) of IPSP amplitude (obtained from single sweep data: 60–250 events at -55 mV) was large and in the range of 0.9–3.5 for first ($n = 9$) and 0.5–2 for the second ($n = 9$) IPSPs. The largest CVs correspond to the smallest IPSPs, which included a large proportion of failures.

SCA interneurons connections are sensitive to CB1 receptor activation

As shown in Fig. 3, SCA interneuron IPSPs were sensitive to the CB receptor agonist anandamide ($14 \mu\text{M}$), which reduced IPSP amplitudes (paired *t*-test, $P < 0.05$, $n = 6$) and increased the number of apparent failures of transmission. The CB1 receptor antagonist AM-251 ($5 \mu\text{M}$) reversed the action of anandamide. The average IPSP amplitude after bath application of anandamide was $50.5 \pm 13.7\%$ of control (paired *t*-test, $P < 0.05$, $n = 6$). Subsequent addition of AM-251, restored the IPSPs to $97 \pm 2\%$ of control ($n = 3$). Figure 3*D* illustrates the change in IPSP amplitudes after bath application

of anandamide and AM-251 for individual pairs. The decreases in failure rate after bath application of anandamide for individual pairs is shown in Fig. 4*C*.

SCA interneuron connections exhibit DSI

During the post DSI protocol (depolarization of the postsynaptic membrane, see Fig. 4), the mean amplitude of the initial IPSPs was reduced to $0.25 \pm 0.09 \text{ mV}$ compared with pre DSI amplitude $0.5 \pm 0.2 \text{ mV}$ (reduced to $50 \pm 11.3\%$, $P < 0.05$, $n = 3$). This reduction in the IPSP amplitudes was accompanied by an increased rate of apparent failures of synaptic transmission (Fig. 4*C*, paired *t*-test, $P < 0.05$, $n = 3$). Repeating the DSI protocol after the addition of AM-251, prevented the effects of DSI and the IPSP amplitudes were restored to $95 \pm 3.5\%$ ($n = 3$) of control values (Fig. 4).

The IPSP amplitude distributions of one connection in control, during DSI, and after bath application of AM-251 + DSI are illustrated in Fig. 4*Ad*. The amplitude distribution is relatively evenly distributed about the mean in each condition.

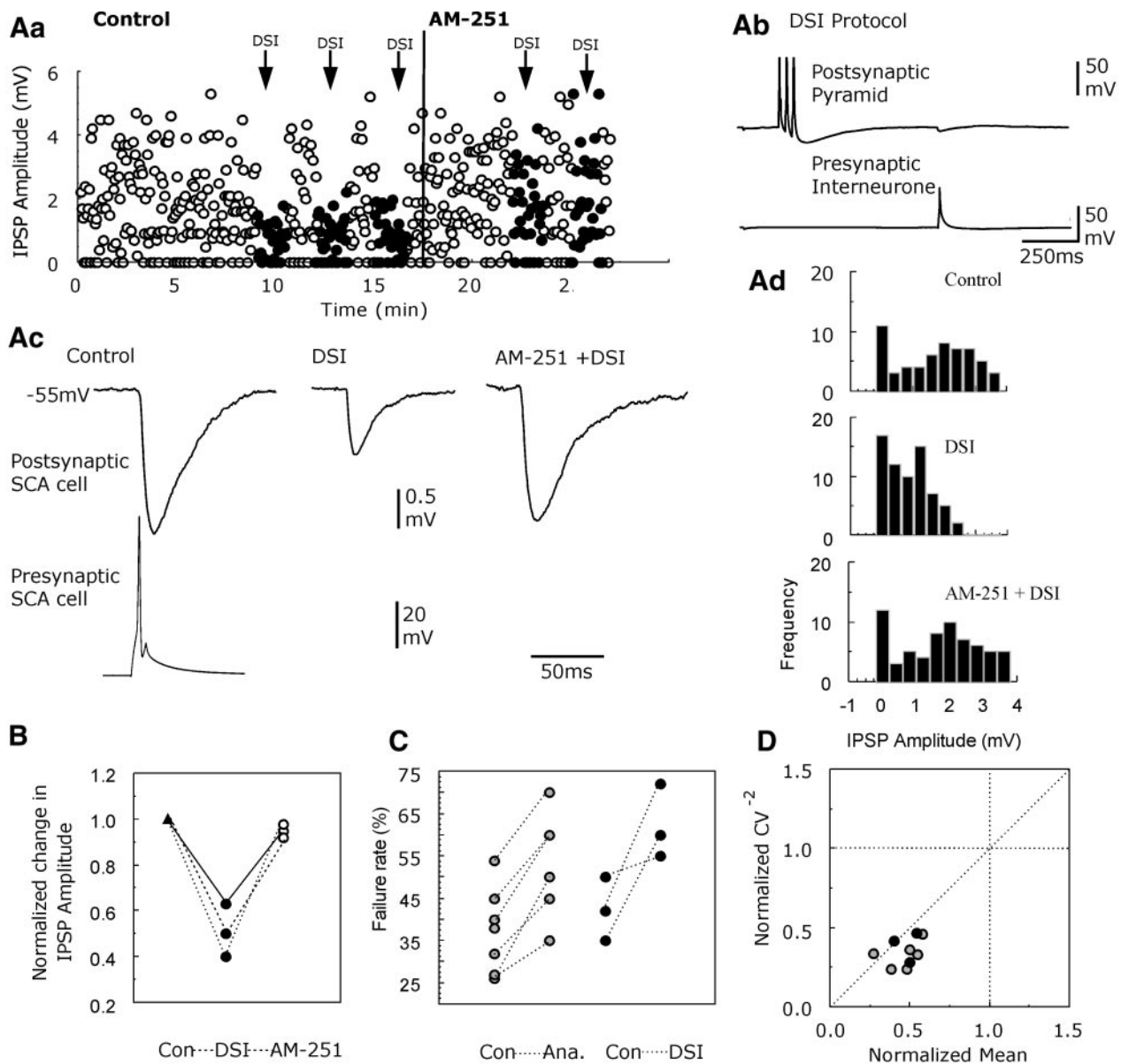


FIG. 4. IPSPs elicited in SCA interneurons display depolarization suppression of inhibition (DSI), which is presynaptically mediated. *Aa*: plot of the peak IPSP amplitude during the time course an experiment in control condition (\circ) and during bath application of AM-251 (\bullet); \downarrow , where DSI was induced (schematic of this protocol shown in *Ab*). \bullet , IPSP amplitude during the DSI protocol in control and AM-251. *Ac* and *Ad*: average IPSPs and amplitude distribution in control condition, during DSI, and during bath application of AM-251 + DSI from the experiment illustrated in *Aa*. The DSI protocol reduced the IPSPs by 50%, which was prevented by AM-251 (data from individual pairs are shown in *B*). There was a shift in the IPSP amplitudes toward smaller values in addition to an increased failure during the DSI protocol. *C*: plot of the average failure rates in control and after addition of either anandamide or during DSI for individual pairs, IPSP failure rate increased during both anandamide and during DSI. *D*: plot of normalized CV^{-2} against normalized mean for IPSPs elicited by SCA interneurons after anandamide and DSI. The majority of the points fall on a slope >1 , indicating a presynaptic origin for the change in the mean after the application of anandamide or during DSI.

Presynaptic sites mediate the effects of CB1 receptor agonist and DSI

To determine whether the change in IPSPs during anandamide and DSI were due to presynaptic mechanisms, a simple binomial model of synaptic release was assumed. In a this simple binomial model of synaptic release, comparisons of changes in the inverse square of coefficient of variation (CV^{-2}) and mean amplitude (M) may indicate whether the depression of unitary IPSPs observed was of pre- or postsynaptic origin; an increase in q (quantal amplitude), results in an equivalent proportional increase in M , $[npq]$, but no change in CV^{-2}

[equivalent to $np/(1-p)$], where n is equal to the number of release sites, and p , the probability of release. A change in n alone results in an equivalent proportional change in both M and CV^{-2} , whereas a change in p results in a greater proportional change in CV^{-2} than in M . Figure 4*D* is a plot of normalized CV^{-2} (test CV^{-2} /control CV^{-2}) against normalized M (test IPSP/control IPSP); the majority of the data points (each point represents a pair), indicate that the change in amplitude of the IPSPs after the application of anandamide or DSI was associated with larger proportional increase in CV^{-2} than in M (slope >1). Therefore the depression of unitary

IPSPs observed is predominantly of presynaptic origin due to a change in p .

DISCUSSION

The present study describes for the first time, the pattern of GABA release between synaptically connected SCA interneurons in the CA1 region of the rat hippocampus and the mechanism by which unitary IPSPs at these synapses are modulated. The IPSPs elicited in postsynaptic SCA interneurons displayed strong synaptic facilitation and augmentation, were sensitive to DSI, and were suppressed by CB1 receptor activation. SCA interneurons were also found to be electrically coupled.

Role of SCA interneurons in the network

The synaptic facilitation observed between SCA interneurons is in striking contrast to the depression typically seen at inhibitory inputs onto pyramidal cells and SP interneurons from several classes of presynaptic interneurons including CCK-basket cells in CA1 (Ali et al. 1998, 1999). It is assumed that when interneurons connect with each other an increased activity of the primary interneuron will lead to an increased firing of the target of the secondary interneuron, referred to as disinhibition (Freund and Buzsáki 1996). Here, CA3 Shaffer collaterals activate CA1 pyramidal cells as well as SCA interneurons, providing a feed-forward inhibition to the dendrites of CA1 pyramidal cells (McBain and Fisahn 2001). This dendritic inhibition to CA1 pyramidal cells will be lifted via the activation of other "primary" SCA interneurons and will depend on their presynaptic firing rates. It can be predicted that depressing inhibitory synapses, e.g., inhibitory inputs to pyramidal cells will be sensitive to small changes in presynaptic firing from high to low rates. However, SCA interneurons which receive facilitating IPSPs will be inhibited or "turned-off" most effectively by small populations of presynaptic SCA interneurons firing at high rates. This disinhibition will allow certain groups of pyramidal cells to continue to report ongoing activity. However, those SCA interneurons that are "turned-off" probably display DSI (via the endocannabinoid system) that will tend to counteract their inhibition by causing a decrease in the presynaptic release of GABA (see following text). These mechanisms will have a significant effect on spike timing of the neurons in this local circuitry and probably play a role in filtering dendritic inhibition to CA1 pyramidal cells in relation to low-frequency theta rhythm.

A significant proportion of the CCK-positive, SCA interneurons were coupled electrically via soma- or dendrodendritic contacts. This provides further evidence to suggest coordinated firing of connected SCA cells that could contribute to the generation of rhythmic synchronous activity. This is consistent with previous observations that CCK-containing interneurons with an accommodating firing pattern synchronize via direct interconnections (Galarreta et al. 2004; Nunzi et al. 1985) or by networks on calretinin-containing interneuron-specific cells coupled electrically or chemically (Freund and Buzsáki 1996; Katona et al. 1999; Tsou et al. 1999). Thus CCK positive SCA interneurons may be responsible for fine tuning oscillatory rhythms in the theta frequency range.

Presynaptic inhibition at GABAergic terminal of CCK-positive SAC interneurons by CB1 receptors

The SCA interneurons studied were immunopositive for CCK. Previously it has been shown that CB1 receptor mRNA is predominantly localized in the presynaptic terminals of subsets neocortical and hippocampal GABAergic interneurons (Irving et al. 2000), particularly CCK-containing basket cells (Hajos et al. 2000; Katona et al. 1999). In the present study, the IPSPs elicited in SCA interneurons were reduced by the CB receptor agonist, anandamide, and this effect was mimicked by depolarization of the postsynaptic membrane (DSI). The effect of the DSI protocol suggests the involvement of a retrograde messenger, most likely to be the endogenous endocannabinoid system in this local inhibitory circuitry. The release of endocannabinoids from the postsynaptic SCA interneuron activates presynaptic CB1 receptors and induces the depression of unitary IPSPs by decreasing GABA release. These results are consistent with previous studies that have implicated endogenous cannabinoids as the retrograde messenger in DSI (Ferraro et al. 2001; Hajos et al. 2000; Katona et al. 1999; Kreitzer and Regehr 2001; Ohno-Shosaku et al. 2001; Paton et al. 1998; Trettel and Levine 2003; Wilson and Nicoll 2001; Wilson et al. 2001; see also Freund et al. 2003 for review), although these studies have used a much more intense conditioning protocol to induce DSI and the release of endocannabinoids from postsynaptic neurons.

In the present study, fluctuation analysis indicated that the suppression of inhibition is most likely to be presynaptic in origin, and this was consistent with the analysis of apparent failures of transmission that decreased significantly during DSI and CB agonist application. The presynaptic activation of CB1 receptors is most likely linked to an inhibition of N- or P/Q-type voltage-gated calcium channels involved in vesicular release (Hoffman and Lupica 2000; Twitchell et al. 1997).

Conclusion and functional implications

This study describes, for the first time, synaptic connections among CA1 interneurons that display synaptic facilitation and augmentation. Unitary IPSPs were modulated by CB1 receptor modulators and DSI. Not only do the dynamics of GABA release and the pre- and postsynaptic mechanisms involved in mediating responses determine the overall effect of synaptic inhibition, but presynaptic mechanisms responsible for modulating inhibition among interneurons may play an important role during synchronous network activity. The chemical and electrical local circuitry of SCA interneurons is an example of how inhibitory connections among interneurons can govern the "clocking" of other interneurons and allow the same class of interneurons to behave differently during different network oscillations (Klausberger et al. 2003).

These CCK-positive interneurons probably play a fundamental role in controlling anxiety and panic. CCK-positive cells relay inputs from diverse cortical and subcortical regions about emotional, motivational, and physiological states (Freund 2003), and polymorphisms in the CCK and CCK-B receptor genes occur in families with inheritable panic disorder (Hosing et al. 2004; Miyasaka et al. 2004). There is evidence to relate CCK basket cells with $\alpha 2/\alpha 3$ GABA_A receptor subtypes (Pawelzik et al. 2002; Thomson et al. 2000) and indeed

$\alpha 2/3$ subunits are thought to mediate anxiolytic actions of benzodiazepines (Atack et al. 2005; Dias et al. 2005; McKernan et al. 2000; Low et al. 2000; Rudolph et al. 1999), suggesting that these CCK cells may play a role in the modulation of anxiety.

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