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Distinctions among GABA_A and GABA_B responses revealed by calcium channel antagonists, cannabinoids, opioids, and synaptic plasticity in rat hippocampus

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Abstract

Rationale—Hippocampal interneurons release γ -aminobutyric acid (GABA) and produce fast GABA_A- and slow GABA_B- inhibitory postsynaptic potentials (IPSPs). The regulation of GABA_B eIPSPs or the interneurons that produce them are not well understood. In addition, while both μ -opioid receptors (μ ORs) and cannabinoid CB1R receptors (CB1Rs) are present on hippocampal interneurons, it is not clear how these two systems interact.

Objectives—This study tests the hypotheses that: (1) all interneurons can initiate both GABA_A and GABA_B inhibitory postsynaptic potentials; (2) GABA_B responses are insensitive to mGluR-triggered, endocannabinoid (eCB)-mediated inhibitory long-term depression (iLTD); (3) GABA_B responses are produced by interneurons that express μ OR; and (4) CB1R-dependent and μ OR-dependent response interact.

Materials and methods—Pharmacological and electrophysiological approaches were used in acute rat hippocampal slices. High resistance microelectrode recordings were made from pyramidal cells, while interneurons were stimulated extracellularly.

Results—GABA_B responses were found to be produced by interneurons that release GABA via either presynaptic N-type or P/Q-type calcium channels but that they are insensitive to suppression by eCBs or eCB-mediated iLTD. GABA_B IPSPs were sensitive to suppression by a μ OR agonist, suggesting a major source of GABA_B responses is the μ OR-expressing interneuron population. A small eCB-iLTD (10% eIPSP reduction) persisted in conotoxin. eCB-iLTD was blocked by a μ OR agonist in 6/13 slices.

Conclusions—GABA_B responses cannot be produced by all interneurons. CB1R or μ OR agonists will differentially alter the balance of activity in hippocampal circuits. CB1R- and μ OR-mediated responses can interact.

Keywords

DAMGO; mGluR; Endocannabinoid; CB1R; CA1; iLTD; SR141716A; Agatoxin; Conotoxin; Paired-pulse ratio

Introduction

The hippocampus hosts a wide variety of GABAergic inhibitory interneurons, which are classified according to their functional, anatomical, or molecular properties (for reviews, see Freund and Buzsaki 1996; Freund 2003; Maccaferri and Lacaille 2003). GABA activates two classes of postsynaptic receptor in the central nervous system. The ionotropic GABA_A receptor-channel conducts Cl⁻ and bicarbonate ions (Bormann 1988). The metabotropic GABA_B receptor principally activates a G-protein coupled, inwardly rectifying potassium (GIRK) channel (Andrade et al. 1986; Bowery 1989). Presynaptically, the GABA_B receptor modulates transmitter release by activating GIRKs and inhibiting voltage-gated calcium channels (VGCCs; Andrade et al. 1986; Nicoll 2004 for review). A persistent question has been whether or not both GABA_A and GABA_B responses are typically induced by a given GABAergic interneuron under normal conditions, or only when there is a high probability of release or a reduction of GABA uptake (e.g., Solis and Nicoll 1992). Whether or not all interneurons can produce GABA_B responses is unknown (see Nurse and Lacaille 1997) and modulation of GABA_B responses is not well understood.

A basic distinction among interneurons is that they release GABA via different classes of VGCCs. Some hippocampal interneurons release GABA only by activation of presynaptic ω -conotoxin GVIA ('conotoxin')-sensitive, N-type VGCCs, and others only by ω -agatoxin IVA ('agatoxin')-sensitive, P/Q-type VGCCs (Lambert and Wilson 1996; Poncer et al. 1997). Lenz and Alger (1999) found that DSI, the retrograde signal process now known to be mediated by eCBs (Wilson and Nicoll 2001; Ohno-Shosaku et al. 2001; Alger 2002, for review), was not affected by agatoxin but was abolished by conotoxin. Wilson et al. (2001) recorded from synaptically coupled, interneuron-pyramidal cell pairs, and showed directly that endocannabinoids (eCBs) inhibit release only from interneurons that release via N-type channels.

Functional distinctions between interneuronal subtypes are less clear than morphological or neurochemical distinctions. It is not fully understood how different interneurons are regulated, or how they interact with each other, although various hypotheses are emerging (e.g., Buzsaki 2002; Gillies et al. 2002; Hefft and Jonas 2005; Glickfeld and Scanziani 2006; Karson et al. 2008). Hoffman and Lupica (2000) showed that the GABA_A (but not the GABA_B) component of an evoked inhibitory postsynaptic current was sensitive to an exogenous cannabinoid agonist, while both components were sensitive to the selective μ OR agonist, [D-Ala², NMe-Phe⁴, Gly-ol⁵]-enkephalin (DAMGO). Conceivably, CB1-expressing interneurons activate only GABA_A receptors, while CB1R-lacking interneurons activate both receptors. In the hippocampus, opioids and cannabinoids seem to be independent of each other (Neu et al. 2007), although activation of μ OR causes widespread disinhibition in CA1 (Nicoll et al. 1980; McQuiston and Saggau 2003).

Functional interactions between conotoxin- and agatoxin-sensitive interneurons have recently been described (Karson et al. 2008). If functional classes of interneurons correspond to CB1R-expressing, or μ OR-expressing interneurons, this would be important, as there are many different reports that cannabinoids and opioids show a bidirectional interaction in some behavioral experiments (see Maldonado and Rodriguez 2002 for review). Little is known about the cellular substrates of opioid–cannabinoid interaction, although work on the nucleus

accumbens (Schoffelmeer et al. 2006) and cultured cells suggests that μ OR-CB1R heterodimers could be responsible (Rios et al. 2007). Interactions among groups of interneurons that are selectively sensitive to inhibition by either cannabinoids or opioids should also be considered.

We have taken a pharmacological and electrophysiological approach to investigating possible functional distinctions among hippocampal interneurons, in particular to the regulation of their GABAergic responses. Specifically, we tested the hypotheses that: (1) all interneurons are capable of initiating both GABA_A and GABA_B IPSPs, (2) GABA_B responses are insensitive to mGluR-triggered, eCB-mediated iLTD, (3) GABA_B responses are produced by interneurons that express μ OR, and (4) CB1R-dependent and μ OR-dependent responses interact. We report that GABA_B responses are produced by interneurons that release GABA via presynaptic N-type VGCCs but that they are insensitive to suppression by eCB-mediated iLTD. They are sensitive to suppression by a μ OR agonist, suggesting that μ OR-expressing interneurons are a source of GABA_B responses. Hence, GABA_B IPSPs are not produced by all types of interneurons. We also describe for the first time an interaction between μ OR and CB1R in the regulation of iLTD that affects only a functionally distinct set of interneurons.

Materials and methods

All animal handling protocols were approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine. After the 5- to 7-week-old male Sprague–Dawley (Charles River) rats were deeply sedated with isoflurane and decapitated, we removed the hippocampi and cut them into 400- μ m-thick slices in ice-cold bath solution using a Vibratome model 1000 (Technical Products International, St. Louis, MO, USA). The slices were kept at room temperature for >1 h in an interface holding chamber filled with humid 95% O₂–5% CO₂ before transfer to the submersion-type experimental chamber (Nicoll and Alger 1981) at 30°C.

We recorded from CA1 cells with sharp electrodes (80–150 M Ω) pulled from 1.2 mm o.d. fiber-filled pipettes containing 3 M KCH₃SO₄ inserted into a holder containing 3 M KCl (pH_i=7.2). Responses were recorded with an Axoclamp 2B (Axon Instruments) and data were filtered at 1 kHz, digitized at 4 kHz with a Digidata 1322A (Axon Instruments), and analyzed with pClamp 9.0 routines (Clampex and Clampfit). The bath perfusion included, in mM: 120 NaCl, 3 KCl, 25 NaHCO₃, 1 NaH₂PO₄, 2.5 CaCl₂, 2 MgSO₄, and 20 glucose (pH_o=7.4). 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX, 10 μ M) and *D*-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5, 20 μ M) were also added to block ionotropic glutamate receptors. Evoked inhibitory postsynaptic potentials (eIPSPs; Fig. 1a) were elicited by 100- μ s extracellular stimuli delivered with concentric bipolar stimulating electrodes in stratum (s.) radiatum at 0.05 Hz. Inhibitory long-term depression (iLTD) was induced chemically (10-min bath application of the mGluR agonist, (S)-3,5 dihydroxyphenylglycine (DHPG; see Chevaleyre and Castillo 2003) or evoked by synaptic stimulation (two 1-s/100 Hz stimulus trains given 20 s apart, or four 1-s/100-Hz stimulus trains each given 1 s after a 1-s depolarizing dc injection, the trains given 20 s apart).

Analysis

In some experiments, the GABA_B eIPSP was isolated by adding 20 μ M bicuculline to the perfusion solution. To compare the differential effect of various treatments on GABA_A and GABA_B eIPSPs when this was not done, we used two approaches:

- a. Subtraction of the CGP 35348-sensitive component. Adding the selective GABA_B receptor antagonist CGP 35348 to the bath at the end of an experiment left the pure GABA_A eIPSP. Subtracting the GABA_A response from the combined GABA_A–

GABA_B eIPSP, left the GABA_B eIPSP. If iLTD had been induced, then the GABA_A eIPSP isolated with (3-aminopropyl) (diethoxymethyl) phosphinic acid (CGP 35348) was scaled to its control value and subtracted from the control GABA_A–GABA_B eIPSP to obtain the control GABA_B eIPSP.

- b. Estimation and subtraction of the GABA_A component. When it was not possible to add CGP 35348 at the end of the experiment, we extrapolated a single exponential decay function from the peak of the GABA_A eIPSP through the decay phase of the GABA_A–GABA_B eIPSP. Subtraction of this estimated GABA_A response from the GABA_A–GABA_B eIPSP gave the estimated GABA_B response. An example is shown in Fig. 1a and b.

The validity of these methods was confirmed by comparing the kinetics of the derived GABA_B eIPSP from the pure-evoked GABA_B response obtained in the presence of bicuculline. The responses were indistinguishable (cf. Figs. 1b and 2b).

Materials

NBQX disodium salt, APV, (S)-3,5-DHPG, (S)-(+)-a-Amino-4-carboxy-2-methylbenzeneacetic acid (LY 367385), 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP), *N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM 251), and ω -conotoxin GVIA were obtained from Tocris (Ellsville, MO); ω -agatoxin TK was obtained from Peptides International. *N*-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716A) was a generous gift of the National Institute on Drug Abuse and was provided by K. Gormley. All other drugs were obtained from Sigma-Aldrich (St. Louis, MO, USA). Drugs were prepared as stock solutions in water and used at 1/1,000 dilution, except for AM251 and SR141716, which were dissolved in dimethyl sulfoxide (DMSO; final concentration of DMSO in the bath was 0.01%). All drugs were applied by bath-application; the flow rate in the chamber was 0.3–0.5 ml/min. Pretreatment of slices (>1 h) with ω -agatoxin GVIA, AM251, or SR141716 was carried out in the incubation chamber described above. SR141716 or AM251 were used interchangeably to block the cannabinoid receptor. ω -agatoxin GVIA and ω -agatoxin TK were used interchangeably to block P/Q type Ca²⁺ channels. The group I mGluR agonist, DHPG, was applied at 50 μ M, which is a functionally maximal concentration in our system.

All group data are represented as mean $\pm$ SEM. Statistical tests [Student's *t* tests and one-way analysis of variance (ANOVA)] were done in Sigma Plot 8 (Systat Software). The presence of iLTD was determined by a significant ($p<0.05$) suppression of eIPSP 20 min after the washout of DHPG or 20 min after synaptic stimulation. Exponential decay curves were fit to the data in Clampfit 9 (Axon Instruments).

Results

Regulation by N- or P/Q-types of VGCCs

All experiments were carried out in the s. radiatum region of CA1. We found that conotoxin perfusion, 250 nM, reduced the peak GABA_A response to 38.8 $\pm$ 2.29% of its baseline value (Fig. 1c, $n=7$), as was reported (Lambert and Wilson 1996; Poncer et al. 2000). The GABA_B response was reduced to 19.2 $\pm$ 1.27 of its baseline (Fig. 1a and b, see “Materials and methods” for methods of GABA_A and GABA_B component separation). The difference in maximal suppression of GABA_A and GABA_B responses is highly significant ($p<0.001$). The GABA_B component also declined somewhat more rapidly than the GABA_A response, the half-times for depression being 12.9 $\pm$ 0.10 and 13.4 $\pm$ 0.79 min, respectively ($p<0.05$). Agatoxin reportedly reduces evoked GABA_A IPSP/Cs by 50–70% (e.g., Lenz and Alger 1999; Wilson et al. 2001),

and in three experiments in which 200 nM agatoxin was washed into the bath (Fig. 2a), we confirmed and extended these results. The GABA_A eIPSP was reduced to $53.2 \pm 1.72\%$ of baseline, and the GABA_B component was reduced to $37.7 \pm 2.96\%$ of baseline by agatoxin. The GABA_B response isolated with bicuculline, 20 μ M, was also reduced to $34.2 \pm 1.31\%$ of baseline ($n=5$; Fig. 2b), thus supporting the validity of the methods used to determine the GABA_B component. As agatoxin is very expensive and its effects are essentially irreversible over the time course of hours (Wheeler et al. 1994), we pretreated slices with 1 μ M agatoxin for >1 h but did not perfuse it in later experiments.

Regulation by μ ORs

DAMGO, the μ OR agonist, reduced both GABA_A (to $58.9 \pm 1.72\%$ of baseline, $n=17$; cells treated with either 100 or 500 nM DAMGO, $p<0.001$, Fig. 3a) and GABA_B components of the eIPSPs (DAMGO 500 nM, $44.9 \pm 2.06\%$ of baseline, $n=5$; Fig. 3b). The difference in suppression of GABA_A and GABA_B responses is significant ($p<0.001$). Interestingly, naloxone restored the GABA_A eIPSP only to $90.8 \pm 1.13\%$ of baseline, and the GABA_B eIPSP only to $87.7 \pm 1.13\%$ of baseline. In both cases, these values are significantly different from their respective controls ($p<0.05$, ANOVA) suggesting the existence of a μ OR-mediated iLTD, although pursuing this possibility was beyond the scope of the present work. We conclude that μ ORs are located on cells that produce GABA_A or GABA_B eIPSPs, but more effectively reduce GABA_B than GABA_A responses.

Regulation by iLTD

Long-term, eCB-mediated modulation of synaptic plasticity affects some GABA_A responses (Chevalleyre and Castillo 2003); however, there is little evidence about whether or not GABA_B responses are similarly regulated. Activation of group I mGluRs suppresses eIPSPs by mobilizing eCBs (Maejima et al. 2001; Varma et al. 2001; Chevalleyre and Castillo 2003). We used the selective group I mGluR agonist, DHPG at 50 μ M, to activate the eCB system in place of the lipophilic CB1R agonists, which are more difficult to apply and remove rapidly in acute brain slices. We first replicated the findings of Chevalleyre and Castillo (2003) and observed that a persistent iLTD was induced either with a 10-min application of DHPG ($78.7 \pm 1.32\%$ of baseline, $n=27$, $p<0.001$, Fig. 4b) or synaptic stimulation protocols (e.g., Fig. 4a): two times 100 Hz synaptic stimulation ($73.5 \pm 2.42\%$ of baseline, $n=5$, $p<0.001$, Fig. 4b) or four times 100 Hz synaptic stimulation plus a depolarizing voltage step (e.g., Fig. 4a; to $83.9 \pm 1.99\%$ of baseline, $n=5$, $p<0.001$, data not shown). The GABA_A eIPSP was subject to iLTD, and this iLTD was mediated by eCBs, as CB1R antagonists, AM 251, 3 μ M, or SR141716A; 3 μ M (Fig. 4c) prevented its establishment ($94.5 \pm 1.84\%$ of baseline, $n=4$). iLTD was also blocked by the group I mGluR antagonists LY 367385, 100 μ M, plus MPEP, 5 μ M, (Fig. 3c; $95.7 \pm 1.68\%$ of baseline, $n=4$), confirming that eCB mobilization is mediated by mGluR I receptors. Unlike Chevalleyre and Castillo (2003), we found that synaptic stimulation given 25 min after DHPG-iLTD induction elicited a further iLTD ($75.5 \pm 1.94\%$ of baseline, $n=5$, $p<0.001$, Fig. 4d) that was comparable to DHPG-iLTD or synaptically induced iLTD alone.

Hoffman and Lupica (2000) reported that the GABA_B response was not affected by the synthetic CB1R agonist, WIN55212-2. However, the overlap between WIN55212-2 sensitivity and eCB responses is imperfect, and eCB-iLTD depends on factors besides simple CB1R activation (Ronesi et al. 2004; Edwards et al. 2006). To determine directly if GABA_B was affected by eCBs and iLTD, we isolated the GABA_B response with 20 μ M bicuculline (e.g., Fig. 5a). We then applied DHPG and observed that there was no iLTD of the GABA_B response ($103.8 \pm 6.00\%$ of baseline, $n=7$, n.s., Fig. 5b; data from control and agatoxin-treated cells pooled, see below) arguing that eCBs do not modulate the GABA_B response.

There were two possible deficiencies with this experiment on isolated GABA_B eIPSPs: (1) There was no positive control showing that the protocols were sufficient to elicit iLTD; and (2) the mechanism of iLTD is not known, and unknown circuit effects involving a GABA_A response could play a role. Hypothetically, blocking GABA_A receptors would prevent GABA_B- iLTD induction.

To test the susceptibility of GABA_B responses to iLTD while avoiding these problems, we did additional experiments without isolating the GABA_B responses with bicuculline. After DHPG-iLTD was induced and DHPG washed out, we used the procedures outlined in “Materials and methods,” to examine the GABA_B eIPSP before and after iLTD induction, and thus compared GABA_A and GABA_B eIPSPs in the same experiment. The GABA_A response was reduced to $67.7 \pm 3.71\%$ of baseline ($p < 0.001$), and the GABA_B response was reduced only $97.3 \pm 5.46\%$ of baseline by the iLTD induction protocol (n.s., $n=11$, Fig. 5c; for display purposes the data are shown in Fig. 5c as percent eIPSP reduction, rather than percent baseline eIPSP).

Are CB1R and μ ORs expressed on the same types of interneurons?

To determine if CB1Rs and μ ORs are expressed on the same functionally defined interneurons, we examined the effects of DAMGO in the presence of either conotoxin or agatoxin. As shown in Fig. 6, 500 nM DAMGO reduced the agatoxin-insensitive GABA_A response (to $64.6 \pm 1.95\%$ of baseline, $n=5$, $p < 0.001$) and the conotoxin-insensitive GABA_A response (to $36.2 \pm 2.50\%$ of baseline, $n=4$, $p < 0.001$). There was a significant difference between the DAMGO effects on agatoxin- and conotoxin-insensitive eIPSPs not only during application ($p < 0.001$, t test) but also after applying naloxone 5 μ M ($102.4 \pm 2.85\%$ of baseline in agatoxin against $88.4 \pm 3.07\%$ baseline in conotoxin, $p < 0.05$, t test).

Ca²⁺ channel subtypes and eCB-mediated iLTD

After agatoxin pretreatment, a 10-min application of DHPG induced iLTD, indicating that P/Q channels are not required for the GABA_A responses that are susceptible to iLTD, or for the iLTD induction process (Fig. 7a agatoxin, $73.1 \pm 4.56\%$ of baseline, $n=8$, $p < 0.001$, t test).

We then asked if P/Q-type VGCCs were important for modulation of GABA_B receptors by DHPG. There was no difference between the DHPG effects on the pharmacologically isolated GABA_B eIPSP recorded in agatoxin and on the GABA_B eIPSPs in non-agatoxin-treated cells (to $106.1 \pm 8.88\%$ of baseline, $n=4$, n.s., agatoxin-treated cells; compared to $94.4 \pm 2.67\%$ of baseline in control cells, $n=3$; control and agatoxin-treated cells pooled in Fig. 5b).

Despite the large suppression of the GABA_A response by conotoxin (Fig. 1), there was a small but significant iLTD (Fig. 7b) of the remaining GABA_A eIPSP after a 10-min exposure to DHPG in conotoxin in five of seven cells tested (to $74.2 \pm 3.05\%$ of the eIPSP remaining in conotoxin, cf. Fig. 1, $n=5$, $p < 0.001$). This was unexpected in view of previous evidence that conotoxin occluded the effect of CB1R agonists and abolished DSI (Lenz et al., 1998; Wilson and Nicoll 2001). Two obvious possible explanations for the residual iLTD were that: (1) it is not mediated by CB1R, or (2) that it is mediated by CB1R receptors on terminals that do not release GABA by N-type VGCCs. To determine which, we repeated the experiment in the presence of the CB1R antagonist AM251, 3 μ M (plus conotoxin). The residual iLTD was blocked by AM251 ($94.9 \pm 4.46\%$ of baseline, $n=3$, n.s., Fig. 7c).

μ OR effects on CB1-mediated iLTD

Whether μ OR and CB1 systems interact at the level of long-term inhibitory synaptic plasticity is not known, although prior results point to an independence of the interneurons that express the two kinds of receptors (Neu et al. 2007). Nevertheless, most experiments have not directly

tested for interactions. We applied DHPG for 10 min in the presence of DAMGO to determine if eCB actions are altered when μ ORs are also activated.

We observed two different effects: In 46% (6/13) of the cells, the presence of DAMGO increased the DHPG-induced eIPSP suppression to $69.5 \pm 3.36\%$ of baseline ($p < 0.01$) compared with a reduction to $79.7 \pm 1.63\%$ in control solution, and completely blocked the iLTD normally caused by DHPG (to $104.57 \pm 2.48\%$ of baseline, n.s., $n=6$). In other cells, DHPG increased eIPSP suppression (to $60.4 \pm 1.98\%$ of baseline) and yet normal iLTD was observed (to $77.5 \pm 1.59\%$ of baseline, $n=7$, $p < 0.001$). The differences between the groups were highly significant (Fig. 8a and b). Such divergent results could mean that different groups of interneurons were primarily activated in the two cases. In fact, we observed that the paired pulse ratios (PPRs) of the remaining GABA_A eIPSPs were differentially affected by DAMGO depending on whether iLTD was observed or not. DAMGO shifted the PPR of non-iLTD-responsive eIPSPs (Fig. 8a) significantly from 0.79 ± 0.04 to 1.00 ± 0.04 ($p < 0.001$), i.e., there was no longer any paired-pulse depression in DAMGO. In contrast, DAMGO did not alter the PPR of iLTD-responsive eIPSPs (0.85 ± 0.04 to 0.89 ± 0.01 , Fig. 8b). DHPG had no lasting effect on PPR in either group, and naloxone reversed the change in PPR for the non-iLTD group (Fig. 8c).

Discussion

The vast amount of information about hippocampal interneurons has led to a number of generalizations about functional properties of different interneurons and their regulation. We have tested certain hypotheses regarding interneuronal responses and classes of interneurons and provide new information that not only fills in some gaps in the literature but also leads to new questions. The major findings of our study address two separate but related issues: regulation of GABA_B eIPSPs and regulation of iLTD.

The source of GABA_B eIPSPs has been unclear, and one possibility was that the GABA_B system could be activated by all interneurons under certain conditions. A prediction of this hypothesis is that the GABA_B eIPSPs should be equally susceptible to blockade by VGCCs that inhibit GABA_A eIPSPs. Conotoxin reduced the GABA_A eIPSP by 61% and agatoxin reduced it by 47%. In contrast, conotoxin reduced the GABA_B response by 81%, and agatoxin reduced it by 62%, suggesting that, unlike the GABA_A responses, the GABA_B eIPSPs originate to a significant extent from interneurons using both N- and P/Q-type channels. Hoffman and Lupica (2000) reported that GABA_B eIPSPs were not affected by application of the synthetic CB1R agonist, WIN55212-2, suggesting that they are not produced by the CB1R-expressing interneurons, 97% of which also express the neuropeptide, cholecystokinin (CCK; Katona et al. 1999). We now report that the GABA_B eIPSPs are not altered by eCBs and are therefore not generated by CCK-interneurons. Neurogliaform interneurons are the source of GABA_B eIPSPs in neocortex, where they principally target dendrites (Tamas et al. 2003). In CA1, neurogliaform neurons communicate with one another via GABA_B synapses (Price et al. 2005), although this network is located in s. lacunosum-moleculare, and it is not known if these cells also communicate with the pyramidal cells. Neurogliaform cells lack CB1R receptors and represent likely candidates for the GABA_B eIPSP. It was interesting to note that, although both GABA_A and GABA_B eIPSPs were greatly reduced by conotoxin, there was nevertheless a subtle but significant functional distinction between them: Over the time course of drug exposure, the GABA_B eIPSP was reduced more rapidly than was the GABA_A eIPSP. This heightened sensitivity of GABA_B suggests potential functional differences between the two GABA responses that could be investigated in future work.

IPSP regulation by μ ORs

μ ORs are detected immunohistochemically on parvalbumin expressing (PV)- but not CCK-expressing interneurons, and most μ OR-expressing cells were PV-containing (Drake and

Milner 2002). PV cells release GABA exclusively via activation of P/Q-type VGCCs (Freund 2003; Hefft and Jonas 2005). These observations predict that large perisomatic eIPSPs that are sensitive to inhibition by DAMGO will be greatly reduced in agatoxin-treated slices. In accordance with the prediction, we find that the effects of DAMGO were much smaller in agatoxin. We cannot determine the identity of the N-type releasing interneurons that are inhibited by DAMGO. The SOM-expressing interneurons, a significant fraction of which also express μ ORs (Drake and Milner 2002), might be good candidates.

eCB-mediated iLTD and GABA_B eIPSPs

We show directly for the first time that GABA_B eIPSPs are not susceptible to mGluR-initiated, CB1R-dependent iLTD. This is consistent with the evidence that the GABA_B eIPSPs are not directly inhibited by CB1R activation (Hoffman and Lupica 2000), but differences between iLTD and processes mediated directly by CB1R activation (e.g., Chevaleyre and Castillo 2003; Edwards et al. 2006) meant that insensitivity of the GABA_B response to iLTD could not be assumed. Our data further support the conclusion that the GABA_B eIPSPs are not produced by CB1R-expressing interneurons and, more importantly, imply that iLTD will alter the normal balance between GABA_A and GABA_B eIPSPs and thereby affect neuronal circuit interactions.

iLTD in conotoxin and enhancement of mGluR-initiated eIPSP suppression in agatoxin

Because hippocampal CB1R receptors are expressed by interneurons that release GABA via activation of presynaptic N-type VGCCs (Wilson et al. 2001), we tested the prediction that iLTD would be absent in conotoxin. We observed that a residual eCB-iLTD (10% depression compared to the control eIPSP baseline response) persisted in conotoxin. This is in agreement with a previous report from our laboratory (Varma et al. 2002) that a small proportion of conotoxin-insensitive mIPSCs were blocked by a CB1R agonist. It is likely that the small percentage of CB1Rs on non-CCK interneurons (Katona et al. 1999) accounted for the residual eCB-iLTD. Conceivably, an alternative explanation for the present result would be that CCK-interneurons can release GABA partially via a non-N-type release mechanism, although the published data (e.g., Wilson et al. 2001; Hefft and Jonas 2005) do not support this interpretation.

If indeed, iLTD is expressed mainly at terminals releasing via N-type VGCCs; blocking P/Q channels with agatoxin should have little effect on iLTD expression, and indeed, we observed that agatoxin did not affect the magnitude of iLTD, although variability in the responses could have masked a small change. The initial percentage reduction of GABA_A eIPSPs caused by DHPG was increased in agatoxin because the extracellular stimulation activates both CB1R-expressing and non-CB1R expressing (agatoxin-sensitive) interneurons. A given absolute depression of release from the CB1R-expressing interneurons will represent a greater percentage reduction of the eIPSPs once the non-CB1R population is removed. Similarly, agatoxin enhances the relative magnitude, although not the absolute magnitude, of DSI by blocking interneurons that are not susceptible to DSI (Lenz et al. 1998). Alternatively, the greater suppression of GABA_A eIPSPs in agatoxin could reflect the removal of a presynaptic interaction between different kinds of s. radiatum interneurons, as recently suggested (Karson et al. 2008).

Interactions between CB1R and μ OR regulation of eIPSPs

An important behavioral and neurophysiological issue involves the potential interactions between cannabinoid and opioid systems. For example, CB1R-lacking mice self-administer heroin at levels markedly less than do wild-type mice (Ledent et al. 1999). If the receptors are present on separate interneurons, then one possibility is that the two groups of interneurons may interact only indirectly via their synapses on the pyramidal cells. If the interneurons bearing CB1R and μ OR are independent, then activating one receptor should have no effect on eIPSP suppression caused by the other. We found that in half of the experiments,

pretreatment with DAMGO did not decrease the effects of DHPG (the percentage reduction of eIPSPs by DHPG was actually slightly increased, as expected if CB1R- and μ ORs are present on different groups of interneurons). In these instances, the μ OR and CB1R systems appear to act independently and in parallel.

However, in the remaining cases, DAMGO significantly reduced the suppressive effects of DHPG and completely blocked iLTD. Either there was some interaction between μ OR- and CB1R-expressing interneurons, or DAMGO uncovered a set of interneurons on which CB1R activation has only short-term but no long-term effects. Intriguingly, when DHPG induced iLTD, the PPR of the eIPSPs was less than one, i.e., paired-pulse eIPSP depression was observed. In contrast, when DAMGO prevented iLTD, the eIPSPs were unaffected by paired-pulse stimulation: the PPR was ~ 1 , i.e., there was neither depression nor facilitation. We propose that among the class of μ OR-insensitive, DHPG-sensitive interneurons, there are two groups that can be distinguished by their paired-pulse responsiveness and the ability to undergo eCB-dependent iLTD. To our knowledge, this is the first report of such a distinction among hippocampal interneurons. A focus of future work should be on the modification of CB1R-mediated responses by μ ORs.

Conclusions

We find that GABA_B responses are produced by interneurons that release GABA via N- or P/Q type calcium channels. They are insensitive to eCB regulation but suppressed by μ OR agonists. GABA_A responses are suppressed by both opioids and cannabinoids, and there is evidence that the receptors are both present on a subset of interneurons. In some cases, the influences of opioids and cannabinoids interact.

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Fig. 1.

Conotoxin differentially suppresses GABA_A and GABA_B eIPSPs. **a** A 250-msec negative dc pulse is given at the beginning of the trace to assess passive cell properties. *Arrows* indicate points at which GABA_A and GABA_B amplitude were measured. The *dashed line* shows an exponential curve fitted to the initial falling phase of the GABA_A eIPSP and extrapolated through the total response. **b** GABA_B response obtained by subtracting the GABA_A eIPSP (calib.=1 mV). **c** Comparison of the GABA_A and GABA_B eIPSP reduction after addition of conotoxin to the bath perfusion ($n=7$). *Traces: Gray trace* eIPSP before conotoxin, *black trace* eIPSP after the application of conotoxin. In all figures, the *traces* shown are the average

of ten traces. The conotoxin concentration was 250 nM in all figures. The calibrations for the traces are: *x*-axis, 2 mV; *y*-axis, 200 ms, unless otherwise stated

Fig. 2. Agatoxin suppression of GABA_A and GABA_B eIPSPs. **a** Comparison of the GABA_A and GABA_B eIPSP reduction after addition of 200 nM agatoxin to the bath perfusion ($n=3$). **b** Time course of isolated GABA_B response reduction by agatoxin ($n=5$). *Traces:* Gray trace eIPSP before agatoxin, black trace eIPSP after the application of agatoxin

Fig. 3. DAMGO depresses both responses. **a** Graph showing the time course of the suppression of GABA_A eIPSPs by DAMGO (100 or 500 nM; $n=17$). **b** Suppression of the isolated GABA_B response (with bicuculline 20 M, present as indicated). *Gray trace* eIPSP before DAMGO, *black trace* eIPSP after the application of DAMGO

Fig. 4. iLTD of GABA responses. **a** Example of a 1-s/100-Hz stimulus train; four were given, each 1 s after a 1-s depolarizing dc injection. **b** A 10-min DHPG application (black circles, $n=27$) reduces the amplitude of an eIPSP for more than 30 min (iLTD). Synaptic stimulation (white circles, two 100-Hz stimulus trains, 20 s apart, $n=6$) also induced robust iLTD. **c** iLTD is blocked by AM 251, 3 μ M (black circles, $n=4$) and by LY367385 100 μ M plus MPEP 5 μ M (gray circles, $n=4$). **d** Synaptic stimulation after a 10-min exposure to DHPG caused a further decrease in the GABA_A response ($n=5$). Traces (Fig. 4a-c): light gray line control, black line 25 min after the application of DHPG or synaptic stimulation. Traces (Fig. 4d): light gray

line control, *dark gray line* 25 min after the application of DHPG, *black line* 25 min after synaptic stimulation

Fig. 5. The GABA_B component of the eIPSP is unaltered by DHPG-mediated endocannabinoid mobilization. **a** Representative GABA_B response before (*black trace*) and 25 min after (*gray trace*) DHPG treatment (calib.=1 mV). **b** The isolated GABA_B is insensitive to a 10-min DHPG treatment ($n=7$); **c** percent reduction in GABA_A and GABA_B eIPSPs by DHPG application

Fig. 6.
Differential effects of calcium channel antagonists on DAMGO-induced eIPSP suppression.
DAMGO suppresses GABA_A eIPSPs to a greater extent in slices treated with conotoxin (*white circles, n=4*) than in slices preincubated in agatoxin (*black circles, n=4*)

Fig. 7. Differential effects of calcium channel antagonists on eCB-induced eIPSP suppression. **a** Pre-incubation with agatoxin does not reduce iLTD. *White circles* Control (not preincubated in agatoxin, $n=27$; same data as in Fig. 3b, *white circles*). *Black circles* Agatoxin, $n=8$). **b** Conotoxin does not entirely prevent iLTD induction measured 20 min after DHPG washout ($n=5$). **c** DHPG-induced iLTD is blocked by AM251 $3 \mu\text{M}$ ($n=3$; calib. for inserts in **b** and **c**=1 mV). Traces before (gray) and after (black) DHPG application

Fig. 8.

DAMGO treatment prevents DHPG-induced iLTD induction in a subset of slices. **a** Pooled data ($n=6$) of cells that did not show DHPG-induced iLTD in the presence of DAMGO. **b** Pooled data of cells ($n=7$) that did show DHPG-induced iLTD in the presence of DAMGO. **c** PPR comparison of cells that did (white bars) and those that did not (black bars) express iLTD in the presence of DAMGO. PPRs are shown for the groups in control solution (con), after adding DAMGO, after washing DHPG from the chamber (wash) and 10 min after adding naloxone (NAL, data not shown). PPR of eIPSPs in DAMGO differs from its value in control and naloxone conditions for the non-iLTD group, as indicated (* $p<0.05$; ** $p<0.001$).

Representative traces showing paired-pulse responses in control conditions, i.e., in DAMGO before DHPG treatment (*gray*) and 20 min after starting to wash DHPG (*black*)