Parvalbumin-Deficiency Facilitates Repetitive IPSCs and Gamma Oscillations in the Hippocampus

MARTIN VREUGDENHIL, JOHN G. R. JEFFERYS, MARCO R. CELIO, AND BEAT SCHWALLER

1Department of Neurophysiology, Division of Neuroscience, Medical School, University of Birmingham, B15 2TT, Birmingham, United Kingdom; and 2Institute of Histology and General Embryology, University of Fribourg, CH-1705, Fribourg, Switzerland

Submitted 19 July 2002; accepted in final form 19 November 2002

Vreugdenhil, Martin, John G. R. Jefferys, Marco R. Celio, and Beat Schwaller: Parvalbumin-deficiency facilitates repetitive IPSCs and gamma oscillations in the hippocampus. J Neurophysiol 89: 1414–1422, 2003; 10.1152/jn.00576.2002. In the hippocampus, the calcium-binding protein parvalbumin (PV) is expressed in interneurons that innervate perisomatic regions. PV in GABAergic synaptic terminals was proposed to limit repetitive GABA release by buffering of “residual calcium.” We assessed the role of presynaptic PV in Ca$^{2+}$-dependent GABA release in the hippocampus of PV-deficient (PV−/−) mice and wild-type (PV+/+) littermates. Pharmacologically isolated inhibitory postsynaptic currents (IPSCs) were evoked by low-intensity stimulation of the stratum pyramidale and recorded from voltage-clamped CA1 pyramidal neurons. The amplitude and decay time constant of single IPSCs were similar for both genotypes. Under our experimental conditions of reduced release probability and minimal presynaptic suppression, paired-pulse facilitation of IPSCs occurred at intervals from 2 to 50 ms, irrespective of the presence of PV. The facilitation of IPSCs induced by trains of 10 stimuli at frequencies >20 Hz was enhanced in cells from PV−/− mice, the largest difference between PV−/− and PV+/+ animals (220%) being observed at 33 Hz. The effect of IPSC facilitation at sustained gamma frequencies was assessed on kainate-induced rhythmic IPSC-paced neuronal oscillations at gamma frequencies, recorded with dual field potential recordings in area CA3. The maximum power of the oscillation was 138 μV$^2$ at 36 Hz in slices from PV+/+ mice and was trebled in slices from PV−/− mice. PV deficiency caused a similar increase in gamma power under conditions used to study IPSC facilitation and can be explained by an increased facilitation of GABA release at sustained high frequencies. The dominant frequency and coherence of oscillations were not affected by PV deficiency. These observations suggest that PV deficiency, due to an increased short-term facilitation of GABA release, enhances inhibition by high-frequency burst-firing PV-expressing interneurons and may affect the higher cognitive functions associated with gamma oscillations.

INTRODUCTION

The calcium-binding protein parvalbumin (PV) is expressed within specific types of neurons throughout the CNS (Celio 1990), where it is implicated in Ca$^{2+}$ homeostasis. Under resting conditions, the Ca$^{2+}$-binding sites of PV are principally occupied by Mg$^{2+}$ ions, which have to be displaced by Ca$^{2+}$, at a rate determined mainly by the dissociation rate of Mg$^{2+}$. This explains why, despite its high affinity for Ca$^{2+}$, the on-rate of Ca$^{2+}$ binding is slow (Schwaller et al. 1999). The intracellular calcium concentration ([Ca$^{2+}$]i) at rest, or peak [Ca$^{2+}$]i during a Ca$^{2+}$ transient, is not affected by PV (Lee et al. 2000), but PV accelerates the initial decay of [Ca$^{2+}$]i (Schwaller et al. 1999).

PV has been implicated in protecting against pathologically high levels of intracellular Ca$^{2+}$ as judged from the high survival rate of PV-containing hippocampal interneurons in temporal lobe epilepsy (Freund et al. 1992; Kamphuis et al. 1989; Sloviter et al. 1991) or after ischemia-induced neuronal degeneration (Freund et al. 1992; but see Hartley et al. 1996). The physiological role of PV at the cellular and network levels is less clear. PV was reported to be concentrated in synaptic terminals of basket type interneurons in the cerebellum (Kosaka et al. 1993), where it is likely to modulate the Ca$^{2+}$-dependent release of GABA. If the presynaptic Ca$^{2+}$ transient evoked by a single spike is insufficient to trigger release, it may prime the terminal for release on subsequent spikes (Kamiya and Zucker 1994). PV may suppress this “residual Ca$^{2+}$”-dependent facilitation if it effectively buffers the presynaptic Ca$^{2+}$ transient. Indeed paired-pulse suppression of IPSCs in Purkinje cells evoked by activating basket cells containing PV in PV+/+ mice turned into facilitation in the cerebellum of PV-deficient mice (Caillard et al. 2000). Paired-pulse suppression of IPSCs could be restored by the addition of the slow Ca$^{2+}$-buffer EGTA (similar Ca$^{2+}$-binding kinetics as PV) into the basket cell via patch pipette.

In the hippocampus, PV is expressed in a subset of interneurons (Aïka et al. 1994; Freund and Buzsáki 1996; Maccari et al. 2000), most prominently axo-axonic interneurons and a subset of basket type interneurons (Kosaka et al. 1987). PV-containing interneurons selectively innervate the perisomatic region of cells (Gulyás et al. 1993; Ribak et al. 1990) and play a major role in the recurrent inhibitory control of principal cells (Freund and Buzsáki 1996). In addition to building a network by mutual synaptic contacts, PV-containing interneurons form a synucytium throughout the hippocampus by dendro-dendritic gap junctions (Fukuda and Kosaka 2000), which is implicated in mediating inhibition-based coherent gamma rhythms (Tamas et al. 2000). Cortical gamma rhythms are

Address for reprint requests: Martin Vreugdenhil, Dept. of Neurophysiology, Div. of Neuroscience, Medical School, University of Birmingham, Edgbaston, B15 2TT, Birmingham, U.K. (E-mail: m.vreugdenhil@bham.ac.uk.)

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
associated with cognition and sensory processing (Engel and Singer 2001).

In the present study, we focus on potentially physiological roles of PV in the hippocampus. We postulate that PV reduces the Ca$^{2+}$-dependent facilitation of GABA release and that, as a consequence, it will affect the inhibition-based gamma rhythms in the hippocampus. To test this hypothesis, we used PV-deficient mice (Schwaller et al. 1999). As a measure of Ca$^{2+}$-dependent GABA release, we monitored the monosynaptic GABA$\_\text{A}$ergic IPSCs in CA1 principal neurons. To concentrate on intrinsic modulation of GABA release, we have selected experimental conditions that favor low release probabilities (Lambert and Wilson 1994; Thomson 1997) and minimize presynaptic suppression of GABA release. To assess the functional significance of any changes in amplitudes of rhythms of gamma oscillations in CA3.

**METHODS**

**Generation of PV-deficient mice**

The PV-deficient (PV$^{-/-}$) mice used in this study were generated by homologous recombination as previously described (Schwaller et al. 1999). Briefly, targeted embryonic stem cells (E14; derived from 129 Ola Hsd mice) were injected into blastocysts of C57BL/6J mice, and the chimeric offspring mated to C57BL/6J animals. Heterozygous (PV$^{+/-}$) mice used in this study were generated (Schwaller et al. 1999). As a measure of Ca$^{2+}$-dependent GABA release, we monitored the monosynaptic GABA$\_\text{A}$ergic IPSCs in CA1 principal neurons. To concentrate on intrinsic modulation of GABA release, we have selected experimental conditions that favor low release probabilities (Lambert and Wilson 1994; Thomson 1997) and minimize presynaptic suppression of GABA release. To assess the functional significance of any changes in amplitudes of rhythms of gamma oscillations in CA3.

**Electrophysiology**

Adult mice, randomly selected from both groups (PV$^{+/-}$ and PV$^{-/-}$) and from controls, were anesthetized by intraperitoneal injection of a ketamine (75 mg/kg)/medetomidine (1 mg/kg) mixture and then killed by cervical dislocation. The brain was quickly removed from the skull and chilled in ice-cold artificial cerebrospinal fluid (ACSF). The composition of the ACSF was (in mM) 125 NaCl, 3 KCl, 26 NaHCO$_3$, 1.25 Na$_2$HPO$_4$, 2 CaCl$_2$, 1 MgCl$_2$, 10 D-glucose; pH was equilibrated at 7.4 with a 95% O$_2$-5% CO$_2$ gaseous mixture. For the recording of GABA$\_\text{A}$ergic responses, the brain was cut into 400-μm-thick transverse slices, using a Vibroslice (Campden Instruments, Sileby, UK). Slices were transferred to a recording chamber (kept at 33°C), wherein they were maintained at the interface between a warm moist gaseous atmosphere (95% O$_2$-5% CO$_2$) and ACSF, flowing at a rate of 2 ml/min. The ACSF was supplemented with 20 μM 6-nitro-7-sulamoyl-benz[b]quinoloxine-2,3-dione (NBQX), 25 μM d-2-amino-5-phosphonovaleric acid (APV), 1 μM CGP 55845A, 5 μM atropine sulfate, and 5 μM naloxone hydrochloride to isolate GABA$\_\text{A}$ergic responses and minimize presynaptic suppression by GABA$\_\text{A}$ receptors (Davies and Collingridge 1993; Lambert and Wilson 1994), muscarinic receptors (Hajos et al. 2000), and μ opioid receptors (Lambert et al. 1991), respectively. To promote facilitation (Lambert and Wilson 1994; Thomson 1997), the release probability was reduced by increasing the concentration of MgCl$_2$ to 3 mM. Brain slices were allowed to equilibrate for 1 h before the onset of recording. Monosynaptic GABA$\_\text{A}$ergic responses were evoked by electrical stimulation (0.1-ms square pulse), using a constant voltage stimulus isolator (Digitimer, Welwyn Garden City, UK). The stimulus was applied with a bipolar electrode, constructed from a pair of insulated, and intertwined 50-μm-diam nickel/chromium wires (Advent Research Materials, Halesworth, UK), placed in the pyramidal cell layer of area CA1, within 0.1 mm from the recording site. Intracellular current-clamp and single-electrode voltage-clamp recordings were taken from neurons within the stratum pyramidale using sharp pipettes filled with 2 M potassium methylsulphate (tip resistance was 50–70 MΩ) connected to an Axoclamp-2A amplifier (Axon Instruments, Burlington, CA). Impaled cells were first inspected in current-clamp and accepted for recording when the resting membrane potential was at least −55 mV and when the current injection-induced overshooting action potentials. For current-clamp recordings, the resting membrane potential was manually adjusted to −65 mV. Single-electrode voltage-clamp recordings were accepted when the switching rate was >4 kHz and voltage-clamp efficiency was >90%, as judged from the difference in the inhibitory postsynaptic potential (IPSP) amplitude between voltage- and current-clamp recordings. The holding potential was −65 mV. Single- and double-pulse stimulations were applied at 15-s intervals, and stimulus trains (10 pulses) were applied at 3-min intervals.

For the recording of network oscillations, 400-μm-thick horizontal slices of the ventral hippocampus were cut and transferred to an interface slice recording chamber where they were allowed to equilibrate for 1 h in standard ACSF at 33°C before the onset of recording. Flow rate was increased to 3–4 ml/min. Extracellular field potential recordings were made with glass pipettes filled with ACSF (tip resistance: 2–4 MΩ) from the pyramidal cell layer of area CA3b/c. Intracellular current-clamp recordings were made from pyramidal neurons in area CA3b/c using methods described in the preceding text.

NBQX, APV, naloxone hydrochloride, and atropine sulfate were obtained from Tocris-Neuramin (Bristol, UK), PV4064 from Swant (Bellinzona, Switzerland), CGP 55845A was a gift from Ciba Geigy (Basel, Switzerland), and all other drugs were purchased from Sigma (Poole, UK).

Signals were low-pass filtered at 3 kHz and sampled at a rate of 10 kHz using a CED 1401 interface and Signal software (Cambridge Electronic Design, Cambridge, UK). Current traces were digitally filtered off-line. The power of the oscillations was measured by...
performing fast Fourier transformations over five consecutive 10-s traces. Cross-correlation analysis between field potentials was made over five consecutive traces, using Spike2 software (Cambridge Electronic Design).

Data are expressed as means ± SE. Unless otherwise indicated, statistical comparisons were made between experimental groups, using unpaired Student’s t-test. The significance criterion was $P < 0.05$.

**RESULTS**

$PV^{-/-}$ mice are not distinguishable from wild-type litter mates

The life span, growth, and breeding of $PV^{-/-}$ mice did not differ from those of wild-type (PV+/+) and heterozygous (PV+/−) animals (Schwaller et al. 1999); the three genotypes were likewise indistinguishable with respect to behavior and physical activity under standard housing conditions. Light microscopic analysis of hematoxylin/eosin-stained brain sections revealed no histological differences between PV−/− and wild-type animals.

**Basket cells in PV−/− mice manifest no PV immunoreactivity**

The B4 lectin from VVA interacts with N-acetylgalactosamine residues alpha-linked to serine or threonine residues in cell-surface glycoproteins. The vast majority of VVA-labeled cells within the hippocampal formation are known to be GABAergic and to express PV (Drake et al. 1991), and in this respect, they resemble previously described VVA-labeled neurons in cerebral cortex (Lüth et al. 1992). Thus VVA staining was used as a means of visualizing, in PV−/− mice, the neuronal subpopulation that would normally express PV. Parallel sections were stained either with a PV-specific antiserum or with peroxidase-conjugated VVA. Images taken from the hippocampus of a wild-type mouse are depicted in Fig. 1. These reveal the presence not only of PV-positive cell bodies but also of a diffuse staining within the s. pyramidale, which demonstrates site specificity of the synaptic terminals (Freund and Buzsáki 1996). As expected, sections derived from PV−/− mice remained unstained after incubation with the PV antiserum, but distribution and number of VVA-labeled neurons was unchanged. For the quantification, only cells with intact somata localized in the s. pyramidale and adjacent regions (s. oriens, s. radiatum) were counted. The number of perineuronal net-positive cells per millimeter pyramidal layer length was $6.61 ± 0.66$ ($n = 3$) for PV+/+ and $6.29 ± 0.31$ ($n = 3$) for PV−/− mice. There was no significant difference between the two groups ($P = 0.69$). At higher magnifications, no differences in the structure of the perineuronal nets were apparent in PV−/− animals (data not shown). Preservation of the perineuronal nets around the cortical neurons of PV-deficient mice has likewise been demonstrated by fluorescence microscopy using the *Wisteria floribunda* agglutinin (Haunso et al. 2000). In both, PV−/− and PV+/+ mice, the perineuronal extracellular matrices were revealed as lattice-like structures around cell soma and dendrites. We conclude that the cell bodies of interneuron types that contain PV in wild-type animals were likewise present and of normal appearance in PV−/− mice but lacked this Ca$^{2+}$-binding protein. Given the absence of a band of VVA staining in s. pyramidale in both groups, we cannot make any predictions on the number or distribution of functional synaptic terminals in PV−/− mice.

**Single inhibitory postsynaptic responses are not affected by PV deficiency**

The most accurate way to investigate the presynaptic role of PV would consist of paired recordings of unitary IPSCs elicited by an interneuron that normally would express PV, like in cerebellar basket cells (Caillard et al. 2000). In the hippocampus, however, it is currently impossible to identify with certainty those interneurons normally expressing PV in wild-type mice, when recording from slices of PV−/− mice. Thus we recorded monosynaptic GABA$\text{\textsubscript{A}}$ergic IPSCs evoked by stimulation of the near s. pyramidale. Under our experimental conditions, the postsynaptic responses were completely blocked by 20 μM bicuculline methiodide and therefore mediated by GABA$\text{\textsubscript{A}}$ receptors (data not shown). In current-clamp mode, the maximal IPSP was determined using stimuli of stepwise increasing amplitude. The maximal IPSP peak amplitude did not differ between the groups ($−14.5 ± 0.7$ mV for 20 cells out of 8 PV+/+ mice; $−13.3 ± 0.8$ mV for 20 cells out of 7 PV−/− mice) and was reached at $30 ± 1$ V for PV+/+ and at $33 ± 1$ V for PV−/− (n.s.). The underlying IPSC was recorded in the same cells at the maximal IPSP stimulus intensity by switching to single-electrode voltage-clamp mode (Fig. 2A). At this stimulus intensity, the GABA$\text{\textsubscript{A}}$ergic IPSC showed a late current reversal, probably due to intracellular chloride accumulation and consequent depolarization of the reversal potential of the GABA$\text{\textsubscript{A}}$ergic, mixed chloride/bicarbonate conductance (Davies and Collingridge 1993; Kaila 1994). The peak amplitude was not different between the
The charge carried by the conditioned IPSC (normalized to the charge of the conditioning IPSC) was maximally facilitated at 3–5 ms and maximally suppressed at 200–500 ms (Fig. 3B). Paired-pulse modulation of IPSCs did not differ between PV+/+ and PV−/− at any interval tested (Fig. 3B). The lack of a change in paired-pulse modulation of IPSCs suggests that the presence of PV does not significantly affect the presynaptic calcium transient induced by a single stimulus. The paired-pulse IPSC suppression at long intervals (Lambert and Wilson 1994; Pearce et al. 1995) may be due to unblocked presynaptic receptors, such as presynaptic metabotropic glutamate receptors (but see Hefft et al. 2002) and cannabinoid receptors (Hajos et al. 2000); but this suppression was unaffected by the lack of PV.

**PV deficiency enhances frequency-dependent facilitation of IPSC trains**

To ascertain whether PV had an effect on prolonged or repetitively induced Ca2+ transients within the presynaptic terminal, we delivered trains of 10 stimuli. The gradual build-up of the intracellular Ca2+ concentration ([Ca2+]i) in the presynaptic terminal during repetitive stimuli is likely to depend on the frequency of stimulation, similar to the situation previously observed in fast-twitch muscles (Schwaller et al. 1999). Thus trains were delivered at different frequencies (0.1,
1, 10, 20, 33, 50, and 100 Hz). At 1 and 10 Hz, IPSC suppression occurred. At frequencies >20 Hz, IPSCs gradually built up with successive stimuli and attained a steady level within 10 stimuli (Fig. 4A). For this cell, the total charge carried by 10 IPSCs at 33 Hz was 188% of that carried by 10 IPSCs at 0.1 Hz. The relative IPSC amplitude measured 10 ms after the stimulus for the nth IPSC is given in Fig. 4B for both groups. At frequencies >20 Hz IPSC facilitation was stronger in seven cells from 6 PV−/− mice than in nine cells from 8 PV+/+ mice. The relative difference increased with the number of stimuli and was significant only after four to five stimuli. This difference could not be explained by a slower IPSC decay because the time constant of current decay did not differ between the groups. Figure 4C gives the facilitation/suppression (mean amplitude of the last 5 IPSCs normalized to that of the first) for each group as a function of frequency. Repetitive IPSC facilitation at frequencies >20 Hz was significantly higher in cells from PV−/− mice than in those from PV+/+ mice, with the difference at 33 Hz (220%) being relatively greater than that at 100 Hz (46%).

Inhibition-based gamma oscillations are facilitated by PV deficiency

In the hippocampus, GABAergic interneurons are involved in coherent network oscillations at frequencies in the gamma band (>30 Hz), which are driven by rhythmic IPSCs (Penttonen et al. 1998; Whittington et al. 1995). The hippocampus-wide network of mutually interconnected PV-containing interneurons (Fukuda and Kosaka 2000) forms a likely substrate for inhibition-based gamma oscillations. Given that the greatest differences in repetitive IPSC facilitation were observed at gamma frequencies, our prediction was that inhibition-based gamma oscillations would be affected in PV−/− mice. Assuming that GABAergic terminals from PV-containing interneurons in area CA3 are similar to those in area CA1 (G. Buzsaki, personal communication), we tested this in vitro using the kainate model of inhibition-based gamma oscillations. At submicromolar concentrations, kainate selectively depolarizes interneurons above firing threshold (Cossart et al. 1998), facilitates GABA release between interneurons (Cossart et al. 2001), and induces persistent oscillations that are driven from area CA3 (Hajos et al. 2000; Traub et al. 2000). Extracellular field potential recordings showed that bath-applied kainate (100 nM) caused robust oscillations in area CA3 (Fig. 5A, top). Current-clamp recordings from CA3 pyramidal neurons showed rhythmic IPSPs with amplitudes within the same range as the IPSPs evoked in CA1 neurons by low-intensity stimulation (Fig. 5A, bottom). Simultaneous recordings demonstrated that the extracellular field positivities in s. pyramidale coincide with the on-going phase of the IPSP (Fig. 5B) and that their peak amplitudes correlate with the IPSP amplitudes (Fig. 5C) and therefore most likely reflect the perisomatic population IPSCs (Buhl et al. 1998). Pharmacologically induced increases and decreases of IPSC amplitude resulted in increases and decreases, respectively, of the amplitude of carbachol-induced gamma oscillations in vitro (Stenkamp et al. 2001). The amplitude of the gamma oscillation can therefore be used as a measure of rhythmic IPSC amplitude. Because the maximum power (~35 Hz) was highly variable within animals, power spectra were calculated from 10 slices from both ventral hippocampi for each animal. Maximum power of the kainate-induced oscillation was approximately three times higher in 70 slices from seven PV−/− mice than that in 70 slices from seven PV+/+ mice (412 ± 53 vs. 138 ± 26 μV^2, P < 0.0001). The averaged power spectra from both genotypes revealed the power of PV−/− slices to be three to four times larger than that in slices from PV+/+ mice across the full range of the gamma frequency band (Fig. 5D). The dominant frequency was not different (35 ± 1 vs. 36 ± 1 Hz) between genotypes. For slices with a maximum power >10 μV^2, there was no relationship between dominant frequency and power (R = 0.03).

Dual field potentials recordings (0.5 mm apart) in slices with clear gamma oscillations showed that kainate-induced gamma oscillations
oscillation under these conditions was 479 ± 53 μV² for PV−/− and 192 ± 47 μV² for PV+/+, \(P < 0.02\) (Fig. 5E).

Therefore under both “normal” conditions and low release probability/minimal presynaptic suppression conditions, the kainate-induced inhibition-based oscillations were strongly enhanced in the absence of PV. Small-amplitude gamma oscillations were occasionally observed even in normal ACSF prior to kainate administration. The average power from 30 to 40 Hz was 2.0 ± 0.5 μV² for PV−/− and 0.7 ± 0.1 μV² for PV+/+, \(P < 0.01\). Thus even without kainate, gamma oscillations were more prominent in slices from PV−/− mice.

**DISCUSSION**

The principal results of this study are that PV deficiency in hippocampal interneurons leads to an increased IPSC facilitation with repetitive stimulation, consistent with greater use-dependent build-up of [Ca²⁺], in the presynaptic terminals of PV-deficient interneurons, and consequently to stronger inhibition-based gamma rhythms.

The staining with VVA-peroxidase complex demonstrates the presence of neurons that would normally express PV (Drake et al. 1991) in the hippocampus of PV−/− mice, whereas staining for PV confirmed that they were devoid of PV. These neurons are likely to make functional synaptic contacts with their normal targets because the IPSC amplitude and kinetics were not different between genotypes; the distribution of the GABA<sub>A</sub> receptor subunit α1, a typical marker for hippocampal PV-expressing basket cells (Klausberger et al. 2002), was not different between genotypes (J.-M. Frischy, personal communication); the distribution of the GABA<sub>A</sub> receptor subunit α2, present at synaptic contacts between axo-axonic PV-expressing cells (Nusser et al. 1996) and the initial oscillations were tightly phase-locked, although amplitudes could vary for each cycle (Fig. 6A). Despite significant differences in power of the oscillation, cross-correlation analysis showed no differences between the groups (cross-correlation coefficient was 0.65 ± 0.05 at 1.1 ± 0.3-ms phase difference for 9 slices from 7 PV−/− mice vs. 0.67 ± 0.04 at 1.0 ± 0.2 ms for 10 slices from 7 PV+/+ mice). Spatial synchronization of kainate-induced gamma oscillations did not depend on the power of the gamma oscillation (Fig. 6B).

To reproduce accurately the experimental conditions used to quantify facilitation of IPSCs, we also analyzed the kainate-induced gamma oscillations in the presence of CGP 55845A, atropine, naloxone, and elevated MgCl₂. This treatment shifted the dominant frequency toward lower frequencies (~30 Hz) in both genotypes and maximum power of the kainate-induced oscillation under these conditions was 479 ± 53 μV² for PV−/− and 192 ± 47 μV² for PV+/+, \(P < 0.02\) (Fig. 5E).

**FIG. 5.** PV-deficiency facilitates kainate-induced gamma oscillations. A: kainate (100 nM) induces high-frequency network oscillations in the hippocampal CA3 region. Extracellular field potential recording from s. pyramidal of CA3b/c (top) shows rhythmic positivities and intracellular current-clamp recording from a pyramidal neuron near the extracellular electrode (bottom) shows rhythmic IPSPs. Cells fired (slightly depolarized) on a minority of the cycles. B: field positivities coincide with the downstroke of IPSPs and action potentials precede the field positivity. C: the amplitude of the field positivities correlates with the amplitude of the IPSP (data from a 5 s recording from the cell in B), indicating that the extracellular oscillation reflects population IPSCs. D: average power spectrum of the oscillation induced by 100 nM kainate in 70 slices from 7 PV−/− mice (black line) and in 70 slices from 7 PV+/+ mice (gray line). PV-deficiency facilitates gamma (>30 Hz) power by 3- to 3-fold compared with values determined in wild-type mice.

**FIG. 6.** PV-deficiency does not affect coherence of gamma oscillations. A: field potentials simultaneously recorded in s. pyramidal of CA3 (0.5 mm apart) show tight phase-locking of events but also a large variability in the amplitude. B: cross-correlation analysis for 9 slices from 7 PV−/− mice (a) and 10 slices from 7 PV+/+ mice (c) showed no differences in phase difference. The maximum cross-correlation coefficient as a function of maximum power of the gamma oscillation showed no relation with the maximum power in the gamma frequency band.
segment of pyramidal cell axons was not different between genotypes (J.-M. Fritschy); and the functional connectivity between cerebellar basket cells and Purkinje cells was not different between genotypes (Caillard et al. 2000). The theoretical contributions to the effects observed in PV−/− mice of either anatomical changes in the terminals of “PV neurons” innervating the recorded pyramidal cells, or of subtle alterations in the presynaptic machinery induced by the PV-deficiency, cannot be excluded and are currently under investigation. Because it is currently impossible to specifically identify those hippocampal interneurons normally expressing PV in wild-type mice in slices from PV−/− mice, we used mono- 
synaptic GABAergic IPSC in CA1 principal cells as a measure of Ca$^{2+}$-dependent GABA release. Mild stimulation of the s. pyramidale activates a mixed population of GABAergic terminals, approximately half of which are PV positive (Ribak et al. 1990). Therefore differences between the genotypes detected using this method, most likely underestimate the PV-deficiency-related changes in PV-expressing synaptic terminals.

Presynaptic PV, as a slow calcium buffer like EGTA, is likely to have little effect on unconditioned transmitter release (Adler et al. 1991). Indeed, unconditioned monosynaptic IPSCs were not different in cells from PV-deficient mice. This is consistent with the lack of an effect of PV on basal [Ca$^{2+}$], levels (Lee et al. 2000; Schwaller et al. 1999), Ca$^{2+}$ currents (Chard et al. 1993), and the expression of GABA$A$ receptors subunits (J.-M. Fritschy).

In normal ACSF, paired activation of putative basket cells and axo-axonic cells results in (presumably presynaptic) paired-pulse suppression of IPSP/Cs in CA1 pyramidal neurons (Ali et al. 1999; Buhl et al. 1995). Under the conditions of reduced release probability (Lambert and Wilson 1994; Thomson 1997) and minimal presynaptic suppression used in this study, the IPSCs exhibited paired-pulse facilitation at short intervals, similar to that found for CA3 neurons (Lambert and Wilson 1994). It is likely that this short-term facilitation results from residual Ca$^{2+}$ in the synaptic terminal (Kamiya and Zucker 1994). As a slow-onset Ca$^{2+}$ buffer, PV does not affect the peak amplitude of the Ca$^{2+}$ transient but accelerates its initial decay (Lee et al. 2000; Schwaller et al. 1999). Given the slow kinetics of the buffer (Schwaller et al. 1999), we predicted therefore that the reduced Ca$^{2+}$ buffering and subsequent accelerated build up of presynaptic [Ca$^{2+}$]i, in the absence of PV (Lee et al. 2000) would favor paired-pulse facilitation of GABA release at intervals between 20 and 300 ms. Indeed, Caillard et al. (2000) demonstrated, for intervals between 30 and 100 ms, increased paired-pulse facilitation of the basket cell triggered IPSC in Purkinje cells from PV−/− mice. However, under our conditions, the paired-pulse IPSC facilitation was not enhanced in CA1 neurons from PV−/− mice. This suggests that compared with the cerebellum, the presynaptic terminals of hippocampal PV-interneurons have either lower net stimulus-induced Ca$^{2+}$ influx and/or a more efficient Ca$^{2+}$ extrusion. Alternatively, the PV concentration in cerebellar basket cells may be higher than in hippocampal basket cells.

The predicted enhanced facilitation of GABA release in cells from PV−/− mice only became apparent in the hippocampus after repetitive stimulation at high frequencies, which will result in a progressive build-up of presynaptic [Ca$^{2+}$]i, if the interval between stimuli is shorter than the [Ca$^{2+}$]i relaxation time constant as modeled for the effect of PV in neurons by Lee et al. (2000). An analogous process of accelerated Ca$^{2+}$ build-up occurred in fast-twitch muscles of PV−/− mice, which had a faster build-up of tetanic tension when stimulated at frequencies >20 Hz (Schwaller et al. 1999). The slow exogenous Ca$^{2+}$ buffer EGTA has a similar effect on excitatory postsynaptic potentials (EPSPs) (Adler et al. 1991; Hochner et al. 1991). The effect of PV was maximal at ~33 Hz for IPSCs and ~20 Hz for tension build-up in fast-twitch skeletal muscle (Schwaller et al. 1999). The limited role of PV at higher frequencies can be explained by its limited buffer capacity. Once all Ca$^{2+}$-binding sites are occupied, any additional Ca$^{2+}$ influx will increase [Ca$^{2+}$]i, and output (GABA release or force) becomes independent of the presence of PV (Lee et al. 2000; Raymakers et al. 2000).

The maximal effect of PV on repetitive IPSC facilitation in CA1 neurons was observed at frequencies in the gamma frequency band (30–80 Hz). Coherent gamma oscillations are associated with higher cognitive processing (Engel and Singer 2001) and are most probably driven by rhythmic IPSCs (Penttonen et al. 1998). In vitro gamma oscillations induced in the hippocampus by metabotropic glutamate agonists (Whittington et al. 1995) in area CA1 and by carbachol (Fisahn et al. 1998) or kainate (Hajos et al. 2000; Traub et al. 2000) in area CA3, are likewise paced by fast GABAergic IPSCs. The hippocampus-wide network of mutually interconnected PV-containing interneurons (Fukuda and Kosaka 2000) is likely to play a major role in mediating inhibition-based gamma oscillations. Assuming that GABAergic terminals in area CA3 are similar to those in area CA1 (G. Buzsaki, personal communication) and given that rhythmic IPSP amplitudes in CA3 neurons were of comparable amplitude as IPSPs evoked at low stimulus intensity in CA1 neurons, we predicted that the absence of PV would increase the amplitude of the rhythmic IPSCs during gamma oscillations. Indeed, the power of both spontaneous and kainate-induced gamma oscillations was increased in slices from PV−/− mice under conditions of low release probability/ minimal presynaptic suppression as well as in normal ACSF. This is in line with the increase in gamma power observed in EEG recordings from mice deficient for the Kv3.1 potassium channel highly expressed in PV-containing interneurons and was associated with increased GABA release (John et al. 1999). Further support comes from the finding of a decrease in gamma power on suppression of IPSCs after application of cannabinoid receptor agonist (Hajos et al. 2000). The effect of PV deficiency on the gamma oscillation was not dependent on release probability as determined by presynaptic modulation or by extracellular Mg$^{2+}$ but most likely due to a PV-related change in presynaptic calcium homeostasis.

The absence of a direct relationship between gamma power (representing IPSC amplitude) and dominant frequency was surprising, because Whittington et al. (1995) showed that the frequency of IPSC-paced interneuron gamma oscillations induced by metabotropic glutamate receptor activation increased with IPSC amplitude. However, similar uncoupling of maximal power and dominant frequency was described for gamma oscillations induced by carbachol (Hack et al. 2000) or kainate (Hajos et al. 2000). A model study of Traub et al. (2000) suggested that the frequency of kainate-induced oscillations was determined by a build-up of activity in a network of
electrotonically coupled pyramidal cell axons after a reset by synchronized IPSCs. Conscious information processing has been associated with changes in coherence of gamma oscillations (Engel and Singer 2001; Miltner et al. 1999). In the present study, coherence of the gamma oscillation was not correlated with the oscillation power and was not affected in PV deficient mice despite gross changes in power. A similar absence of a relation between power and coherence of carbachol-induced gamma oscillations was observed in the isolated guinea pig brain (Dickson et al. 2000).

Functional implications

Under normal conditions, the gradual depression of IPSC amplitude with prolonged high-frequency activation of PV-expressing basket type interneurons and/or axo-axonic interneurons (Maccarferri et al. 2000), leads to a steady inhibitory potential in the pyramidal cell (Ali et al. 1999; Buhl et al. 1995). PV thus maintains the strength of a perisomal synapse near its resting level, allowing integration of EPSPs against a steady background of inhibition; this may increase the sensitivity of the integration process.

The power of gamma oscillations increases with attention and recognition in human subjects (Muller et al. 2000) and with sensory information processing in rats (Penttonen et al. 1998). The increase in gamma power in mice deficient for the Kv3.1 potassium channel was associated with better performance in an active avoidance task (Joho et al. 1999). In contrast, the cannabinoid-induced decrease of gamma power in rats (Hajos et al. 2000) may explain the effects of cannabis on cognitive performance. The presence of PV limits the power of gamma oscillations. On the one hand, considering the role of gamma in cognitive functions, this seems like a hindrance. No obvious behavioral changes have been observed in PV−/− mice so far, but more targeted behavioral tasks aimed to specific paradigms (memory, learning) are currently being explored. On the other hand, considering the hyper-synchronous gamma activity at the onset of kainate-induced epileptic discharge (Medvedev et al. 2000), the limitation of the gamma power by PV might be a safeguard against the onset of epilepsy. This is in line with the increased susceptibility toward pentylentetrazol-induced seizures observed in PV−/− mice (Tandon et al. 1999; B. Schwaller, unpublished observation).

We are most grateful to P. Eggli, Institute of Anatomy, University of Bern, for the morphometric analysis and B. Belser, Fribourg for the excellent technical help. The input to the discussion part from J.-M. Fritschy, Institute of Pharmacology and Toxicology, University of Zurich, Switzerland, is highly appreciated. We thank Ciba Geigy for the generous donation of CGP 55845A.

This study was supported by the Swiss National Science Foundation (Grants 3100-047291.96 and 3200-059559.99/1 to M. R. Celio and 3100-063448.001 to B. Schwaller) and the Wellcome Trust.

REFERENCES


