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Regulation of fast-spiking basket cell synapses by the chloride channel ClC-2

Csaba Földy[1,2], Sang-Hun Lee[1], Robert J Morgan[1] & Ivan Soltesz[1]

Parvalbumin-expressing, fast-spiking basket cells are important for the generation of synchronous, rhythmic population activities in the hippocampus. We found that $GABA_A$ receptor–mediated synaptic inputs from murine parvalbumin-expressing basket cells were selectively modulated by the membrane voltage– and intracellular chloride–dependent chloride channel ClC-2. Our data reveal a previously unknown cell type–specific regulation of intracellular chloride homeostasis in the perisomatic region of hippocampal pyramidal neurons.

There are two distinct basket cell classes specialized to provide GABAergic innervation to the perisomatic region of principal cells, the parvalbumin-expressing and cholecystokinin-expressing basket cells (PVBCs and CCKBCs, respectively). The intrinsic and synaptic properties of PVBCs enable them to perform circuit functions related to precise time keeping and generation of network oscillations, whereas CCKBCs are thought to serve as modulators that adapt network activity to behavioral states[1,2]. Because synapses from PVBCs and CCKBCs coexist on the perisomatic membrane, it has been assumed that the regulation of the intracellular concentration of Cl^-, the major charge-carrying anion for $GABA_A$ receptor channels, is uniform at PVBC and CCKBC synapses. Using paired recording techniques[3] in slices (**Supplementary Methods**), we found that the chloride channel ClC-2 robustly modulated synaptic inputs specifically from PVBCs, providing a molecular safety mechanism for preventing the accumulation of intracellular chloride at the highly active GABAergic synapses formed by the fast-spiking PVBCs. Our experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Paired interneuron–pyramidal cell whole-cell patch-clamp recordings showed that the amplitudes of the unitary IPSCs evoked by CCKBCs (CCK-IPSCs) were smaller than those of PVBC-evoked IPSCs (PV-IPSCs) at membrane potentials more depolarized than −35 mV (**Fig. 1a**). On the other hand, below the reversal potential for $GABA_A$ receptor–mediated events (E_{GABAA}), PV-IPSCs were significantly smaller than CCK-IPSCs ($P < 0.05$; **Fig. 1a**). Furthermore, examination of the current-voltage relationships across a wide voltage range (**Fig. 1a**) indicated that CCK-IPSCs exhibited inward rectification (inward current flowed more easily than outward current), whereas PV-IPSCs showed apparent outward rectification.

The origin of the inward rectification of CCK-IPSCs was readily identifiable, as it was a result of depolarization-induced suppression of inhibition[1] that was sensitive to the CB1 receptor antagonist AM251 (10 μM; **Fig. 1a**). However, the difference in amplitude of the inward IPSCs was unexpected, as the number of $GABA_A$ receptor channels is similar at PVBC and CCKBC synapses on CA1 pyramidal cells[4]. To characterize the apparent outward rectification of PV-IPSCs, we

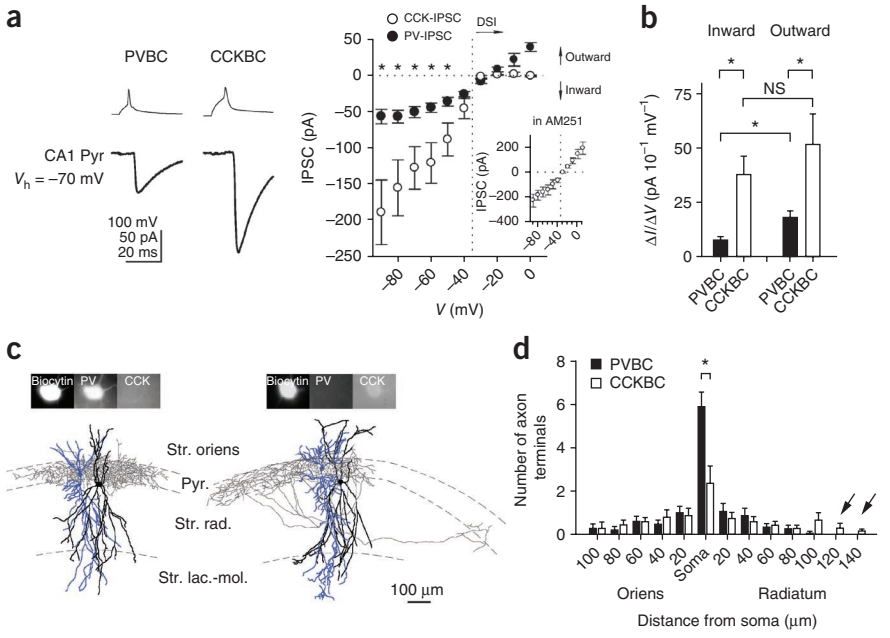

Figure 1 Outward rectification of PV-IPSCs and distribution of PVBC axon terminals on the somata of pyramidal cells. (**a**) Left, averaged example traces (top, presynaptic spikes; bottom, postsynaptic responses; $[Cl^-]_{pip}$ = 48.7 mM). Right, current-voltage plots of IPSCs (failures included; PVBC, $n = 6$ pairs; CCKBC, $n = 5$ pairs; $^*P < 0.05$; NS, not significant; errors are s.e.m.; probability of release was similar between the two groups, see **Supplementary Methods**). Inset, CCK-IPSCs in AM251 ($n = 4$ pairs). DSI, depolarization-induced suppression of inhibition; pyr, pyramidal cell. (**b**) Average $\Delta I/\Delta V$ of the plot in **a** (CCK-IPSCs in AM251). (**c**) Examples of basket cells (axons, gray; dendrites, black) and pair-recorded postsynaptic pyramidal cells (blue). lac.-mol., lacunosum-moleculare; Pyr, pyramidale; rad, radiatum; Str, stratum. (**d**) Distribution of terminals ($P = 0.03$). Arrows indicate distal CCKBC terminals.

[1]Department of Anatomy and Neurobiology, University of California, Irvine, California, USA. [2]Present address: Department of Molecular and Cellular Physiology, Stanford University, Palo Alto, California, USA. Correspondence should be addressed to C.F. (foldy@stanford.edu).

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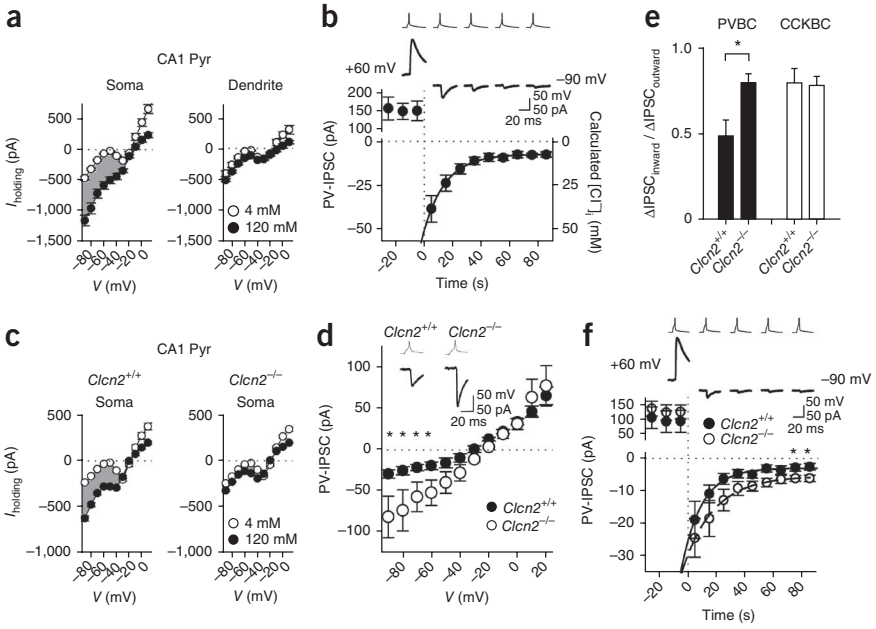

Figure 2 Somatic hyperpolarization-gated, sustained Cl^- conductance mediated by ClC-2. (**a**) Whole-cell recordings from the somata and proximal apical dendrites of pyramidal (pyr) cells with different $[Cl^-]_{pip}$ (4 mM, n_{soma} = 14, $n_{dendrite}$ = 4; 120 mM, n_{soma} = 11, $n_{dendrite}$ = 4; shading indicates the difference current reflecting whole-cell Cl^- current). (**b**) Time-dependent decrease of PV-IPSCs after stepping the membrane voltage of the postsynaptic cell from +60 mV to −90 mV in rat. (**c**) Large sustained somatic Cl^- conductance in somata of CA1 pyramidal cells in the wild-type (*Clcn2*$^{+/+}$), but not the *Clcn2*$^{-/-}$, mice (4 mM, $n_{soma,+/+}$ = 13, $n_{soma,-/-}$ = 20; 120 mM, $n_{soma,+/+}$ = 24, $n_{soma,-/-}$ = 24). (**d**) Current-voltage plots of PV-IPSCs from *Clcn2*$^{+/+}$ (n = 6 pairs) and *Clcn2*$^{-/-}$ (n = 3 pairs) mice. Inset, PV-IPSCs from *Clcn2*$^{-/-}$ mice compared with *Clcn2*$^{+/+}$ mice (example traces at −70 mV, $[Cl^-]_{pip}$ = 40 mM). (**e**) Significantly decreased outward rectification of the PV-IPSCs in the *Clcn2*$^{-/-}$ mice and lack of change in rectification in the case of CCK-IPSCs (P = 0.0005). (**f**) Slower time-dependent decrease of PV-IPSCs after stepping the membrane voltage of the postsynaptic cell from +60 mV to −90 mV in the *Clcn2*$^{-/-}$ mice compared with *Clcn2*$^{+/+}$ mice. Asterisks indicate significant difference (P = 0.02) (note that the larger IPSCs indicated by asterisks in these Cl^- extrusion experiments are consistent with the presence of larger IPSCs at hyperpolarized holding potentials in the *Clcn2*$^{-/-}$ mice in **d**).

compared the inward and outward portions of the current-voltage relationships ($\Delta I/\Delta V$, reflecting synaptic conductance; **Supplementary Methods**). Although the average $\Delta I/\Delta V$ of inward and outward CCK-IPSCs were not different (in AM251, 37.7 ± 8.5 and 51.8 ± 14, n = 4 pairs, P = 0.424), the average $\Delta I/\Delta V$ for inward PV-IPSCs was significantly smaller than that for outward PV-IPSCs (7.6 ± 1.5 and 18 ± 2.9, n = 8 pairs, P = 0.007; **Fig. 1b**). Consequently, the ratio of the average $\Delta I/\Delta V$ of inward versus outward currents (reflecting rectification) was also significantly smaller for the PV-IPSCs (0.43 ± 0.04 versus 0.75 ± 0.06, n = 8 versus n = 4 pairs, P = 0.001).

An explanation for the smaller inward PV-IPSCs compared with the CCK-IPSCs is that the driving force for Cl^- is lower at PVBC than at CCKBC synapses. Indeed, paired recordings with low (4 mM) intracellular Cl^- concentration (close to physiological values[5]) revealed that the difference in inward IPSC amplitude between the PVBC and CCKBC inputs was accompanied by differences in the E_{GABAA} values (PVBC, −70.8 ± 0.9 mV, n = 8 pairs; CCKBC, −67.8 ± 0.9 mV, n = 13 pairs, P = 0.04; **Supplementary Fig. 1**), indicating lower intracellular $[Cl^-]_i$ at PVBC synapses. Such differential regulation of $[Cl^-]_i$ could conceivably occur at the level of individual synapses and/or subcellular domains[5–8]. Because synapses from the two types of basket cells intermingle and are assumed to distribute similarly on perisomatic membranes, we performed a morphological analysis of our recorded pre- and postsynaptic cell pairs (**Fig. 1c**). PVBC axons formed more putative synaptic terminals on the postsynaptic pyramidal cells than CCKBCs (11 ± 0.6, n = 15 pairs versus 8.3 ± 0.8, n = 14, P = 0.02; note, however, that the number of release sites per terminal may differ between PVBCs and CCKBCs; for a review, see ref. 1). In addition to differences in the total number of terminals, the distribution of the terminals in the perisomatic compartment was also different. Namely, PVBCs formed approximately twice as many axon terminals on the soma (5.8 ± 0.7 (n = 15 pairs) versus 2.3 ± 0.8 (n = 14 pairs), P = 0.02), whereas the CCKBC terminals extended farther out onto the apical dendrites (**Fig. 1d**).

The preferential cell type–dependent innervation of subcellular compartments may provide an anatomical basis for a mechanism conveying domain-specific regulation of $[Cl^-]_i$. Indeed, whole-cell recordings with either low (4 mM) or high (120 mM) intra-pipette Cl^- ($[Cl^-]_{pip}$) from the soma or apical dendrite (60–80 µm from soma, close to the middle of the basket cell synapse distribution in the stratum radiatum; **Fig. 1d**) of single CA1 pyramidal cells revealed hyperpolarization-gated, sustained Cl^- conductance preferentially at the pyramidal cell soma (note that these data do not exclude the presence of such a Cl^- conductance elsewhere in the dendritic tree; **Fig. 2a**).

Next, paired recording experiments between PVBCs and pyramidal cells were conducted by first evoking large outward PV-IPSCs at +60 mV (and presumably loading the postsynaptic cell body with Cl^-; $[Cl^-]_{pip}$ = 4 mM) and then stepping the membrane potential to −90 mV. Inward PV-IPSCs immediately after the step to −90 mV were large (−37.8 ± 7.5 pA, n = 5 pairs), but then the amplitude decreased over tens of seconds (τ = 14.4 ± 1.8 s, n = 5 pairs; **Fig. 2b**), consistent with the presence of a mechanism that lets Cl^- ions exit from the inside to the outside according to the Cl^- electrochemical gradient (note that similar Cl^- extrusion experiments with CCK-IPSCs resulted in a significantly slower decrease in the event amplitude after the step to −90 mV, τ = 24.7 ± 3.8 s, n = 5 pairs, P = 0.04).

A mechanism that could potentially underlie these effects is the hyperpolarization-activated, inwardly rectifying plasma membrane Cl^- channel ClC-2 whose gating also depends on $[Cl^-]_i$ (a rise in intraneuronal Cl^- concentration opens ClC-2 and results in an efflux of Cl^-)[9–11]. Both mRNA and protein for the ClC-2 channel are known to be expressed in CA1 pyramidal cells, but not in granule cells of the dentate gyrus[12,13]. Consistent with the lack of ClC-2 expression in granule cells, paired recordings from PVBCs and postsynaptic granule cells revealed no marked outward rectification of the PV-IPSCs and the somatic Cl^- conductance was also lacking in granule cells (**Supplementary Fig. 1**). Furthermore, the somatic Cl^- conductance was not present in CA1 pyramidal cells from mice lacking the ClC-2 channel[14] (*Clcn2*$^{-/-}$; **Fig. 2c**). In addition, paired recordings from PVBCs and postsynaptic CA1 pyramidal cells in mice showed increased inward currents (**Fig. 2d**) and, consequently, significantly

reduced outward rectification of PV-IPSCs in *Clcn2*$^{-/-}$ mice (*Clcn2*$^{+/+}$, 0.42 ± 0.05, n = 8 pairs; *Clcn2*$^{-/-}$, 0.89 ± 0.06, n = 7 pairs; P = 0.0005; **Fig. 2e**). Note that the rectification of the CCK-IPSCs did not change in the *Clcn2*$^{-/-}$ mice (*Clcn2*$^{+/+}$, 0.79 ± 0.08, n = 3 pairs; *Clcn2*$^{-/-}$, 0.78 ± 0.05, n = 4 pairs; P = 0.8; **Fig. 2e**). Finally, Cl^- extrusion experiments (similar to those in rat in **Fig. 2b**) showed a significantly slower decrease in the PV-IPSC amplitude after the step to −90 mV in the *Clcn2*$^{-/-}$ mice (*Clcn2*$^{+/+}$, τ = 14.9 ± 1.1 s, n = 4 pairs; *Clcn2*$^{-/-}$, τ = 22.6 ± 2.5 s, n = 4 pairs; P = 0.03; **Fig. 2f**). Additional experiments showed a significantly longer time to reversal of the inward (depolarizing) IPSCs to outward (hyperpolarizing) IPSCs in CA1 pyramidal cells from *Clcn2*$^{-/-}$ mice than from *Clcn2*$^{+/+}$ mice after a brief period of intense presynaptic GABAergic fiber activity evoked by multi-fiber extracellular stimulation resulting in increased intracellular [Cl^-] (**Supplementary Fig. 1**).

Our data suggest that PVBC synapses are regulated by ClC-2. The ClC-2–mediated selective modulation of PVBC inputs appear to be ideally suited for preventing potentially dangerous rises in $[Cl^-]_i$ (and thus depolarizing $GABA_A$ responses) during episodes of intense synchronized firing during hippocampal network oscillations by populations of fast-spiking PVBCs that form convergent inputs on single pyramidal cells[15] (in contrast, CCKBCs fire at lower frequencies *in vivo*[2]). Unlike several other $[Cl^-]_i$-regulating mechanisms[5], ClC-2 does not influence the resting $[Cl^-]_i$ under normal circumstances when E_{GABAA} is more hyperpolarized than the resting membrane potential. Activation of ClC-2 may also be aided by K^+ conductances (for example, postsynaptic $GABA_B$ receptors) that can hyperpolarize the membrane potential below E_{GABAA} or by extracellular acidification[11]. Future studies will be required to determine whether differential $[Cl^-]_i$ regulation exists even at adjacent synapses from PVBCs and CCKBCs and whether the differential activity of ClC-2 at PVBC inputs is a result of differences in the levels of ClC-2 expression (that is, ClC-2 may exist at some CCK synapses[13]) and/or channel modulation[11].

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

C.F. and S.-H.L. designed the experiments. C.F., S.-H.L. and R.J.M. performed the experiments and analyzed the data. C.F. and I.S. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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