examination. Finally, these animals will serve as useful recipients for the reintroduction of class II molecules targeted to specific locations in order to better understand the role of these proteins in various autoimmune phenomena.

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Excitatory Synaptic Responses Mediated by $GABA_A$ Receptors in the Hippocampus

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Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the cortex. Activation of postsynaptic $GABA_A$ receptors hyperpolarizes cells and inhibits neuronal activity. Synaptic responses mediated by $GABA_A$ receptors also strongly excited hippocampal neurons. This excitatory response was recorded in morphologically identified interneurons in the presence of 4-aminopyridine or after elevation of extracellular potassium concentrations. The synaptic excitation sustained by $GABA_A$ receptors synchronized the activity of inhibitory interneurons. This synchronized discharge of interneurons in turn elicited large-amplitude inhibitory postsynaptic potentials in pyramidal and granule cells. Excitatory synaptic responses mediated by $GABA_A$ receptors may thus provide a mechanism for the recruitment of GABAergic interneurons through their recurrent connections.

STUDIES OF THE CELLULAR AND CIRcuit properties of the hippocampus have focused primarily on the output excitatory neurons, the pyramidal cells. Inhibitory GABAergic interneurons are the other major cell type in the hippocampus (*1*). The features of these inhibitory cells are not as well characterized as those of the pyramidal cells, presumably because it is difficult to obtain intracellular recordings from these sparsely distributed elements (*2*). During normal activity, interneurons are activated by afferent fibers (feedforward inhibition) (*3*) and by recurrent axons of pyramidal cells (feedback inhibition) (*4*). Excitation through these pathways is mediated by glutamatergic synapses. However, recent studies in the cortex (*5*) suggest that another mechanism, independent of glutamatergic transmission, may be involved in the synchronization of GABAergic interneurons.

We investigated the glutamate-independent recruitment of interneurons by recording intracellularly from pyramidal cells and interneurons in the hippocampal slice (*6*). In the presence of the convulsant compound 4-aminopyridine (4-AP) (50 to 100 μM) and excitatory amino acid (EEA) receptor blockers (*7*) [3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), 10 to 30 μM, and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 10 μM], rhythmic, synchronized inhibitory postsynaptic potentials (IPSPs) (*7* to 15 mV peak amplitude; up to 900 ms in duration) occurred in pyramidal and granule cells. These events occurred at frequencies ranging from 0.1 to 0.3 Hz (*8*).

To explore the origin of the synchronized IPSPs, we obtained intracellular recordings from GABAergic interneurons. The hilar region of the dentate gyrus is particularly suitable for such a study. A dense population of GABAergic interneurons is located in an identifiable site that is distinct from the granule-cell layer and devoid of granule-cell dendrites (*9*). In the presence of 4-AP and EAA receptor blockers, intracellular recordings obtained from electrodes placed in stratum pyramidale or within the granule cell layer consistently revealed spontaneous, synchronized IPSPs. In contrast, in a large proportion of intracellular recordings (80%) from neurons within a specific region of the hilus (see Fig. 1), we saw spontaneous, rhythmic bursts of action potentials. Simultaneous recordings indicated that bursts in these hilar cells occurred simultaneously with synchronized IPSPs in principal (pyramidal and granule) cells (Fig. 1A). Recordings from pairs of hilar cells demonstrated that burst firing occurred simultaneously in these neurons (Fig. 1A). Thus, we named the depolarizing events in hilar cells synchronized bursts.

Spontaneous synchronized bursts in hilar interneurons and corresponding synchronized IPSPs were also recorded in the presence of EAA receptor blockers when the extracellular potassium concentration ($[K^+]_o$) was raised from 5 to 12 mM (*10*) (Fig. 1B). Synchronized events recorded in the high $[K^+]_o$ condition occurred sporadically. However, they could be triggered consistently by electrical stimulation applied at frequencies lower than 0.2 Hz (see below).

We visualized hilar neurons that had the above described electrophysiological properties. Recording electrodes containing the fluorescent dye Lucifer yellow (LY) were used, and cells of interest were filled with the dye (*6*). Eleven bursting hilar cells were filled with LY (Fig. 1C). All of the filled cells were nonpyramidal and shared some common morphological features. The somata of these cells were typically ovoid in shape, with dendrites preferentially extending in a plane parallel to the granule cell layer. The shape of these cells resembled that of horizontal cells. Horizontal-type cells in the hilar region are positively stained by antibodies to glutamic acid decarboxylase, the synthetic enzyme for GABA, suggesting that they are GABAergic neurons (*11*).

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We examined the properties of the synchronized events by evoking the responses at various membrane potentials. Hyperpolarization of hilar cells during an evoked event suppressed action potentials and revealed that the burst firing was sustained by a large-amplitude depolarizing synaptic event (Fig. 2A). Intracellular recordings from hilar cells also showed that unitary IPSPs occurred spontaneously in these cells. At the resting potential of about −60 mV, unitary events were hyperpolarizing, whereas synchronized bursts occurring at the same membrane potential were sustained by underlying membrane depolarizations (Fig. 2A). The depolarizing synaptic event in hilar cells did not reverse in polarity when the membrane potential was set to about −30 mV, indicating that this event could depolarize the cell beyond the firing threshold and cause excitation.

For comparison, we examined the relationship between membrane potential and evoked synaptic events (synchronized IPSPs) in a CA3 pyramidal cell. Three peaks along the time course of the synchronized IPSPs could be followed (Fig. 2B). Each of the peaks had a different reversal potential of −74, −65, and −95 mV, respectively. Other studies show that application of GABA elicits three responses on hippocampal cells. One response, reversing at about −95 mV, can be attributed to the activation of $GABA_B$ receptors; the other two, with reversal potentials of about −75 and −40 mV (hyperpolarizing and depolarizing responses, respectively), are mediated by $GABA_A$ receptors (*12, 13*). The three peaks in the synchronized IPSPs may correspond to the depolarizing and hyperpolarizing responses mediated by $GABA_A$ receptors and the hyperpolarization sustained by $GABA_B$ receptors. At the resting membrane potential of −60 mV, synchronized events recorded in principal cells were hyperpolarizing. In contrast, the synchronized events in the hilar cells were depolarizing and excitatory at the resting potential.

Our results indicate that depolarizing synaptic events in GABAergic interneurons synchronized their activity, producing large amplitude IPSPs in principal cells. We were interested in determining which neurotransmitter system was sustaining the hilar neuron burst. The excitatory synaptic events, because they could be recorded in EAA receptor blockers, clearly were not sustained by glutamatergic transmission. In addition, they are not mediated by β-adrenergic or muscarinic cholinergic receptors because the burst events persisted after treatment with propranolol or atropine (Fig. 2C). Two hypotheses, consistent with the data, could explain the origin of the depolarizing synaptic events and how the synchronization of GABAergic interneurons occurred. (i) GABAergic interneurons might be synchronously activated by excitatory projections of a subpopulation of simultaneously firing neurons, as yet unidentified. The excitatory synaptic responses evoked by these hypothetical cells presynaptic to the interneurons are resistant to EAA blockers (*14*). (ii) Some GABAergic synapses formed between interneurons could function as excitatory synapses, allowing GABAergic neurons to excite each other by their recurrent connections. We tested these two possibilities by treating the slice with picrotoxin (*15*). We found that both the burst events and the underlying synchronized excitatory synaptic potentials (EPSPs) in hilar cells were completely blocked by picrotoxin (50 μM) (Fig. 3, A and B). In addition, the unitary IPSPs occurring spontaneously along the baseline were also blocked by the agent (Fig. 3A). In two other experiments, we observed that the addition of bicuculline

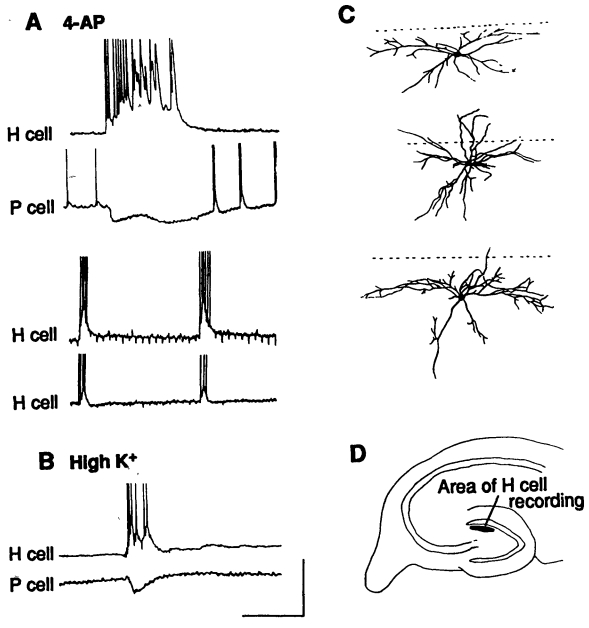

Fig. 1. (**A**) Simultaneous recordings in the presence of 4-AP and EAA receptor blockers. Top two traces illustrate the spontaneous synaptic event in a CA3 pyramidal cell (P cell) and the corresponding burst in a hilar cell (H cell). Bottom two traces are simultaneous recordings from two neurons in the hilar region. Downward deflections in the upper of these two traces were activated by applied pulses of hyperpolarizing current. The amplitude of the pulses indicates the input resistance of the cell. During the burst, input resistance of the cell decreased significantly. (**B**) Simultaneous recordings from a P cell and an H cell recorded 45 min after the concentration of $[K^+]_o$ was raised from 5 to 12 mM. Horizontal bar, 300 ms for top two traces and 1.75 s for bottom two traces in (A), and 450 ms in (B). Vertical bar, 40 mV. Action potentials are truncated. (**C**) Morphology of typical hilar neurons exhibiting synchronized bursting activity. The dotted line represents the inner border of the dentate granule cell layer. The neurons were traced from photographs of the fluorescent image of H cells filled intracellularly with LY. The diameter of the cells was approximately 20 μm. (**D**) Transverse slice of the hippocampus showing the location of H cell recordings.

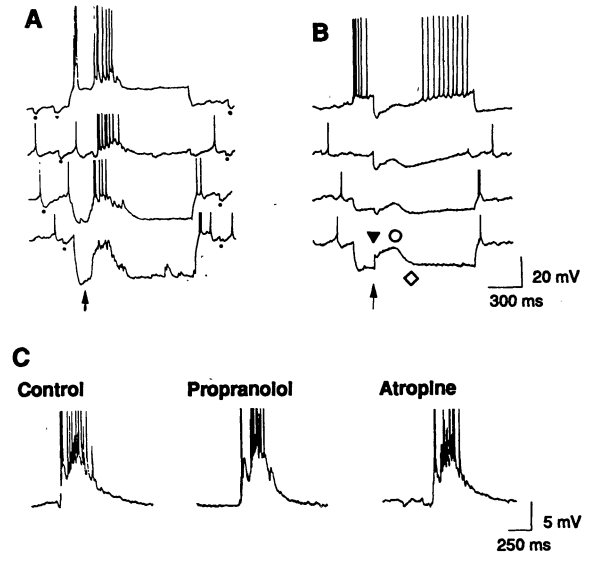

Fig. 2. Effects of membrane potential changes on the evoked synchronized burst in an H cell (**A**) and the giant IPSP in a P cell (**B**). Synchronized bursts were elicited by stimulation of perforant path fibers. The cell was depolarized (top trace) or hyperpolarized (lower two traces) during the evoked event. Arrow, point of stimulus application; dots, spontaneous unitary IPSPs. At resting potential (second trace from the top), about −60 mV, when the unitary IPSPs were hyperpolarizing, a depolarizing potential sustained the synchronized burst. (B) Three peaks occurred along the giant IPSP. Second trace from top was obtained at the resting potential (about −60 mV). Each of these peaks showed a different reversal potential. These peaks occurred at 15 (▼), 210 (○), and 350 (◇) ms after stimulation. The reversal potentials were −74, −65, and −95 mV, respectively. Arrow, point of stimulus application. (**C**) Burst response in an H cell recorded in the presence of 4-AP and EAA blockers before (control) and after addition of propranolol (15 μM) and atropine (1 μM). Bursts were recorded 45 min after addition of drugs to the bath. Propranolol was washed out for 1 hour before atropine was added to the bath.

also blocked synchronized burst firing in interneurons. Bursts in hilar interneurons and corresponding synchronized IPSPs in pyramidal cells evoked in slices bathed in high $[K^+]_o$ (12 mM) were also blocked by picrotoxin (Fig. 3B). These observations demonstrate that the synchronized EPSPs in hilar neurons are mediated by $GABA_A$ receptors.

Two additional observations are consistent with the hypothesis that some GABAergic synapses formed between interneurons can be excitatory. (i) Spontaneous unitary EPSPs and IPSPs were recorded in hilar interneurons in normal extracellular medium after treatment with EAA blockers (Fig. 4A). Addition of picrotoxin (50 μM) blocked both these synaptic events (Fig. 4B), indicating that spontaneous EPSPs and IPSPs were mediated by $GABA_A$ receptors. (ii) Application of GABA by means of pressure ejection to hilar neurons elicited depolarizing responses (Fig. 4C). Together, these data suggest that the activity of interneurons may be synchronized by the recurrent connections of these neurons (Fig. 4D).

This study indicates that GABA can function as an excitatory neurotransmitter to synchronize the firing of inhibitory interneurons in the hippocampus. This synaptic excitation mediated by $GABA_A$ receptors primarily affects GABAergic interneurons. The overall effect of excitatory coupling between inhibitory cells is an enhanced output of the interneuron population, giving rise to giant IPSPs in principal cells. In this way, the excitatory action of $GABA_A$ receptors remains consistent with the role of GABA as an inhibitory neurotransmitter in the signaling process.

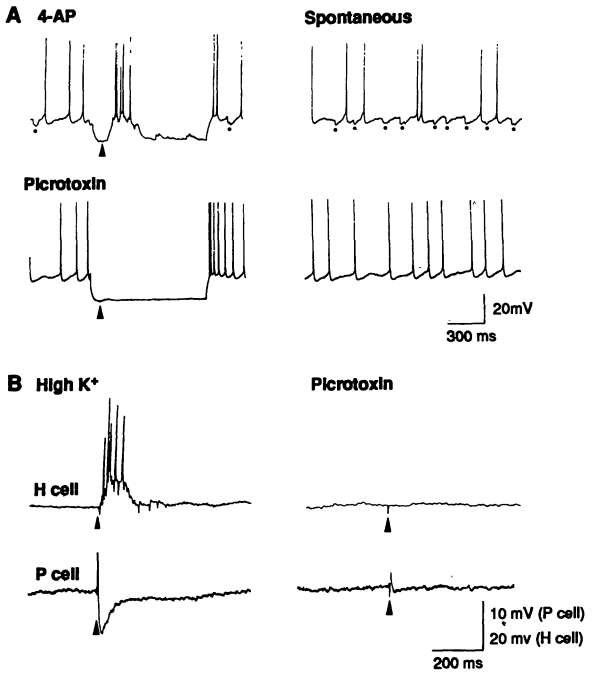

Fig. 3. (**A**) (Top trace, left) An evoked synchronized burst in an H cell during membrane hyperpolarization. Dots, spontaneous unitary IPSPs. (Top trace, right) Spontaneous activity from the same cell as that shown on left. (Lower traces) Same cell as in (A) after treatment with picrotoxin (50 μM). (Left) Synchronized bursts can no longer be elicited (arrowhead, stimulus application). (Right) Picrotoxin also blocked the spontaneous unitary IPSPs. (**B**) (Left) Simultaneous recordings from an H cell and a P cell obtained in elevated $[K^+]_o$ (12 mM) and EAA receptor blockers. Arrowhead, stimulus application. (Right) Same cells as left, recorded 45 min after treatment with picrotoxin (50 μM).

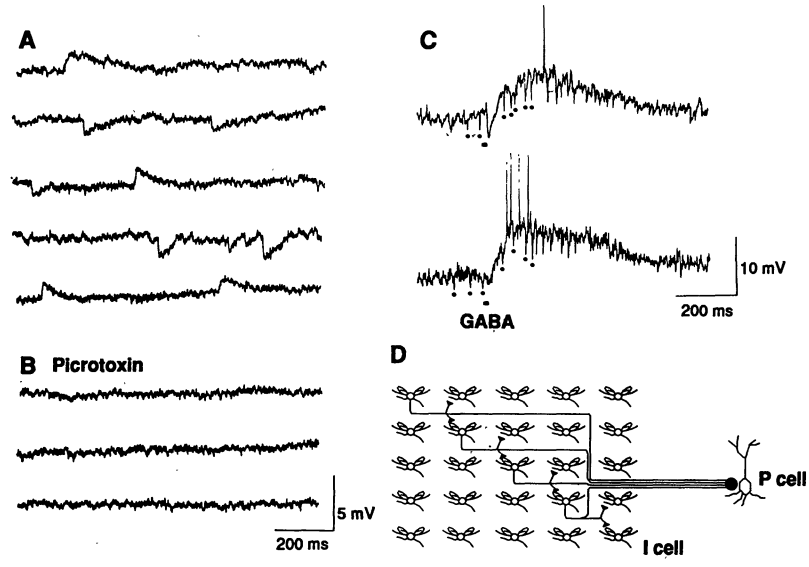

Fig. 4. (**A**) Unitary EPSPs and IPSPs recorded in a hilar neuron in the presence of EAA receptor blockers, 80 min after washing out 4-AP. 4-AP was initially in the bath to facilitate identification of a bursting hilar neuron. (**B**) Same cell as in (A), after treatment with picrotoxin (50 μM). (**C**) Effects of pressure application of GABA on a hilar neuron. Bar, GABA application (15-ms duration, 20 psi). Dots mark some unitary IPSPs. (Top trace) Biphasic hyperpolarizing-depolarizing response to GABA application. (Bottom trace) Moving the pipette containing GABA to another site (10 μm deeper into the slice at the same location) resulted in a depolarizing response to GABA application. (**D**) Schematic diagram of the proposed synchronized inhibitory circuit. Inhibitory (I) cells are mutually excited through their recurrent collaterals; the synchronized output inhibits principal (P) cells. The I cells in the circuit excite at least two postsynaptic I cells. Each postsynaptic cell in turn excites at least two additional cells. A conceptually similar scheme has been proposed for the synchronized discharge of principal cells during an interictal spike; ◀, excitatory terminal; ●, inhibitory terminal (*16*).

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6. Adult guinea pigs were anesthetized with halothane and decapitated, and the hippocampus was removed quickly. Transverse, 400-μm thick slices were prepared in oxygenated 4°C buffer with a vibratome. Slices were then placed in a warm (35.5°C), humidified (95% O_2, 5% CO_2) recording chamber at an interface of air and buffer solution (126 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.6 mM $MgCl_2$, 26 mM $NaHCO_3$, 10 mM dextrose, pH = 7.4). In some experiments the concentration of KCl was increased to 12 mM. Intracellular electrodes were pulled from borosilicate glass (60 to 80 megohms). Electrodes were filled either with 2 M potassium acetate or with 4% LY (Sigma) in 1 M LiCl [W. W. Stewart, *Nature* **292**, 17 (1981)]. To fill cells with the dye, we applied 1- to 3-nA hyperpolarizing pulses of duration 750 ms at 0.67 Hz. In experiments involving application of GABA, glass pipettes (10 megohm) were filled with GABA dissolved in buffer solution to a final concentration of 1 mM. The pipettes were connected to a "picospritzer" (General Valve); GABA was applied by pressure (20 psi, 4- to 20-ms duration) to recorded neurons.
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13. GABA application activates $GABA_A$ receptors and elicits depolarizing and hyperpolarizing responses in the dendrites and somata, respectively, of pyramidal cells [P. Andersen *et al.*, *J. Physiol. (London)* **305**, 279 (1980); B. E. Alger and R. A. Nicoll, *ibid.* **328**, 125 (1982)]. Some studies suggest that both responses are mediated by an increase in Cl^- conductance and the difference in polarity is caused by a difference in intracellular Cl^- concentration in the soma and dendritic segments (*12*). However, other data show that low concentrations of GABA elicit hyperpolarizing responses in dendrites and somata [R. K. S. Wong and D. J. Watkins, *J. Neurophysiol.* **48**, 938 (1982)]. Increasing concentrations of applied GABA elicited the depolarizing response first in the dendrites and then in the soma. These data suggest that the depolarizing and hyperpolarizing responses are mediated by different receptors and that there is a higher density of receptors mediating the depolarizing response in the dendrites than in the soma.
14. The results further suggest that if such a group of presynaptic cells exists, they would excite interneurons without affecting principal cells because application of picrotoxin abolished synchronized IPSPs in principal cells without revealing any residue excitatory events. Large amplitude, synchronized IPSPs are also recorded in a majority of pyramidal cells when low dosages of penicillin or picrotoxin are added to the hippocampus [R. Miles and R. K. S. Wong, *J. Physiol. (London)* **388**, 611 (1987)] or neocortex [Y. Chagnac-Amitai and B. W. Connors, *J. Neurophysiol.* **62**, 1149 (1989)], respectively. These events are probably initiated by the synchronized discharge of a small group of pyramidal cells through their glutamatergic connections, and the synchronized IPSPs arise from the feedback activation of inhibitory interneurons [R. D. Traub *et al.*, *Science* **243**, 1319 (1989)]. This initiation mechanism cannot account for synchronized events reported here because we added EAA blockers at a concentration sufficient to block EPSPs evoked at stimulus strengths that were four times as high as threshold (*5*).
15. If a subset of cells presynaptic to the interneurons were synchronized, driving the interneurons in synchrony to produce a synchronized IPSP, then the addition of picrotoxin to the bath would block the synchronized IPSP by blockade of postsynaptic $GABA_A$ receptors. However, the interneurons would continue to burst. On the other hand, according to the second hypothesis, picrotoxin would block the recurrent excitatory synaptic transmission between the interneurons, thereby blocking the burst event and the postsynaptic synchronized IPSPs in principal cells.
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Negative Regulation of CD45 Protein Tyrosine Phosphatase Activity by Ionomycin in T Cells

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CD45 is a leukocyte-specific, transmembrane protein tyrosine phosphatase (PTPase) required for T cell responsiveness. How the activity of PTPases is regulated in vivo is unclear. Treatment of murine thymocytes and a variety of murine T cell lines with the calcium ionophore ionomycin decreased CD45 PTPase activity. Ionomycin treatment also led to a decreased phosphorylation of serine residues in CD45. These results indicate that increased intracellular calcium modulates CD45 PTPase activity, demonstrating regulation of CD45 PTPase activity in vivo, and also implicate serine dephosphorylation as a possible mechanism.

CD45 (KNOWN AS LC-A, T200, B220, or Ly-5) is a family of high molecular mass glycoproteins ranging from 180 to 220 kD that is expressed exclusively on nucleated hematopoietic cells (*1, 2*). CD45 has a large, conserved cytoplasmic domain that contains two subdomains with similarity to a placental PTPase domain (*3*) and has intrinsic PTPase activity (*4, 5*). PTPases can be classified into two major groups (*6*): cytoplasmic PTPases with one PTPase domain, exemplified by human placental PTPase 1B (*3*), and transmembrane PTPases that generally contain tandem cytoplasmic PTPase subdomains. The transmembrane PTPases have all the hallmarks of receptors analogous to the growth factor receptors of the protein tyrosine kinase (PTK) family. CD45 is a member of this group and may be a cell-surface receptor although its ligand has not been identified.

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Little is known about how PTPases are regulated. The COOH-terminal region of the cytoplasmic T cell PTPase is involved in the regulation of PTPase activity and subcellular localization (*7*). Because CD45 is abundantly expressed on the cell surface, has high specific activity (*8*), and is constitutively active (*4, 5*), it is likely that the CD45 PTPase activity is subject to negative regulation in vivo. We now demonstrate that an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by ionomycin leads to a decrease in CD45 PTPase activity.

When most mouse T cell lines were treated with ionomycin, CD45 PTPase activity decreased 50 to 90%. The mouse T helper hybridoma AODH 7.1 was maximally inhibited at 1 to 2 μM ionomycin (Fig. 1A). The inhibition was reproducible, and in some cell lines, it was observed at concentrations as low as 0.2 μM. This is the concentration range of ionomycin that is required, in the presence of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), for optimal stimulation of T cells for proliferation (*9*).

To distinguish whether ionomycin treatment reduces the specific activity of CD45 PTPase or decreases the amount of CD45 isolated, we immunoblotted immunoprecipitates of CD45 with a rabbit antiserum to the cytoplasmic domain of mouse CD45. No difference was detected in the amount of CD45 present in the immunoprecipitates from treated or untreated cells (Fig. 1B); exposure to ionomycin is not therefore affecting either the solubility or antigenicity of CD45. Cell viability, measured by Trypan blue exclusion, did not decrease during the assay period. Identical results were observed after treatment with another Ca^{2+} ionophore, A23187, implying that the inhibition was not a nonspecific effect of the ionomycin. When T cells were treated with ionomycin in the presence of the Ca^{2+} chelation agent EGTA, CD45 PTPase activity did not decrease, providing further evidence that this effect is due to the Ca^{2+} flux (Fig. 1C). Thus, these results implicate a Ca^{2+} flux as a regulatory event for CD45 PTPase activity.

To determine if inactivation of the CD45 PTPase occurs in normal lymphoid cells, we treated thymocytes or spleen cells with ionomycin and measured the CD45 PTPase activity (Fig. 2A). When thymocytes were treated with ionomycin, PTPase activity was almost completely inhibited; thus, CD45 PTPase activity is regulated by a Ca^{2+} flux in a normal lymphoid cell population. However, ionomycin had little effect on the activity of spleen cells. One possible explanation for this difference in CD45 PTPase regulation between the thymic- and splenic-derived cells is that only a small subpopulation of spleen cells are affected. CD45 PTPase activity could be regulated differently in T cells versus B cells or at different stages of maturation. Ionomycin treatment resulted in reduced CD45 PTPase activity in