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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

most T lymphomas, as well as a number of $CD4^+$ or $CD8^+$ immunocompetent T cell lines, suggesting that CD45 PTPase activity is not restricted to immature T cells. An increase in $[Ca^{2+}]_i$ does not result in a decrease of CD45 PTPase activity in some murine T cell lines, suggesting that Ca^{2+} flux is not sufficient for inactivation of CD45 and that additional factors are involved.

When cells are treated with ionomycin, there is an almost instantaneous influx of Ca^{2+} (*10*); however, the decrease in CD45 PTPase activity was not detectable until about 5 min after ionomycin treatment of thymocytes and was not maximal until 30 to 40 min after treatment (Fig. 2B). Similar results were seen in all the T cell lines tested. The slow kinetics of the ionomycin effect suggests that the decrease in CD45 PTPase activity is not directly due to Ca^{2+} and that the Ca^{2+} flux induces a cascade of events that ultimately result in diminished CD45 PTPase activity. In addition, Ca^{2+} has little to no effect on the PTPase activity when added directly to the assay (*8, 11*). Taken together, these results suggest that the Ca^{2+} flux induces secondary events that lead to CD45 inactivation.

Because the activity of a number of PTKs is known to be regulated by phosphorylation (*12*), the possibility that changes in the phosphorylation of CD45 may be involved in the loss of PTPase activity was examined. Thymocytes were labeled with $^{32}P_i$ and treated with either TPA, ionomycin, or both in combination. CD45 was immunoprecipitated from each of the thymocyte populations, and the immunoprecipitates subjected to SDS–polyacrylamide gel electrophoresis. Phosphorylation of CD45 decreased significantly after treatment with ionomycin alone and with TPA and ionomycin in combination (Fig. 3A). No change in phosphorylation was seen after TPA treatment alone. Immunoblotting of the same filter with antiserum to CD45 confirmed that each of the immunoprecipitates contained similar amounts of CD45 (Fig. 3B). The decrease in phosphorylation correlated with the loss of CD45 PTPase activity because it was not observed in cell lines in which CD45 PTPase activity was not reduced after treatment with ionomycin. Results of phosphoamino acid analysis suggest that the reduced labeling with $^{32}P_i$ must be the result of a decrease in serine phosphorylation because the trace amounts of phosphotyrosine and phosphothreonine in CD45 remain unchanged after ionomycin treatment. Two-dimensional tryptic peptide maps of CD45 from cells metabolically labeled with $^{32}P_i$ and either left untreated or treated with ionomycin indicated that the labeling of two minor phosphopeptides was decreased relative to the others (Fig. 3C); in this experiment, but not others, a smaller decrease in the labeling of the major phosphopeptide was also seen. Thus, ionomycin treatment selectively decreases serine phosphorylation of CD45 at specific sites, which may in turn regulate CD45 PTPase activity. In contrast to published results with human peripheral blood lymphocytes (PBLs) (*13, 14*), we did not observe any effect of TPA treatment on CD45 phosphorylation or PTPase activity in mouse thymocytes, T cell lines, or human Jurkat cells. Although Yamada and co-workers (*14*) reported a decrease in PTPase activity of CD45 isolated from TPA-treated human PBLs, the relative amounts of CD45 immunoprecipitated from TPA-treated and untreated cells were not determined. In addition, when human CD45 is treated with protein kinase C (PKC) in vitro, there is no detectable change in PTPase activity (*8*). PKC is probably not mediating the decrease in PTPase activity because the effect is seen with ionomycin treatment alone but not with TPA.

These data show that CD45 PTPase activity can be regulated by Ca^{2+} in T cells. Modulation of CD45 PTPase activity may occur by this mechanism when T cells are activated with specific antigen or by antibodies to some cell surface antigens, because of the transient increase in $[Ca^{2+}]_i$ that is induced (*15*). CD45 can regulate the tyrosine phosphorylation and the kinase activity of the *src*-related PTK $p56^{lck}$ (*5, 16*). Further, tyrosine phosphorylation is not induced in $CD45^-$ variants of Jurkat cells after activation (*17*). Thus during T cell activation, CD45 may initially dephosphorylate PTKs, such as $p56^{lck}$, to induce an initial surge in tyrosine phosphorylation. Subsequently, CD45 PTPase may be inactivated in response to the Ca^{2+} flux so that tyrosine phosphorylation of secondary substrates can be maintained; as the $[Ca^{2+}]_i$ returns to basal levels, CD45 PTPase activity would be restored allowing further antigen stimulation. This model is consistent with the observation that $CD45^-$ cell lines cannot be activated (*17, 18*) and with our data.

Our results show that CD45 PTPase ac-

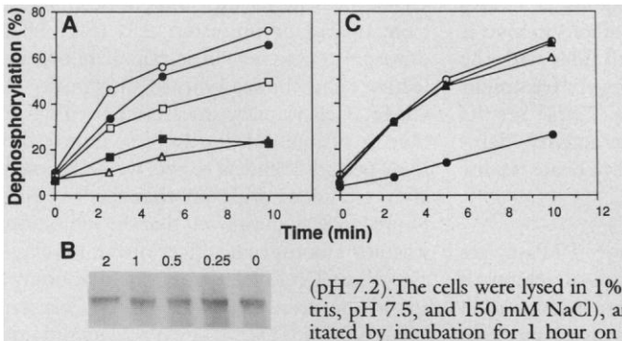

Fig. 1. Ionomycin treatment of cell lines results in decreased CD45 PTPase activity that can be reversed by EGTA. The mouse T helper hybridoma AODH 7.1 (*23*) (5×10^6 cells per milliliter) was treated with ionomycin (>98% pure, Calbiochem) for 20 min at 37°C in Hepes-buffered Dulbecco's modified Eagle's medium (pH 7.2).The cells were lysed in 1% NP-40 in TN buffer (20 mM tris, pH 7.5, and 150 mM NaCl), and CD45 was immunoprecipitated by incubation for 1 hour on ice with I3/2.3 (*24, 25*). The precipitates were washed two times with 0.5% NP-40 in TN, two times with TN alone, and then once with PTPase buffer [25 mM Imidazole (pH 7.2), 5 mM EDTA, and 0.1 mM dithiothreitol]. Equal amounts of the immunoprecipitates (2×10^5 to 5×10^5 cell equivalents) were then assayed for PTPase activity with phosphorylated angiotensin (10^7 cpm/pmol; 4nM) as a substrate (*5*). (**A**) AODH 7.1 cells treated with either 2 μM (△), 1 μM (■), 0.5 μM (□), 0.25 μM (●), or no ionomycin (○). (**B**) Immunoblot of CD45 immunoprecipitates used in (A). The immunoprecipitates were separated by electrophoresis on a 7.5% gel, transferred to Immobilon-P (Millipore), blotted with a rabbit antiserum to the cytoplasmic domain of recombinant mouse CD45 that was produced in a baculovirus system (*2, 5*), and then visualized with a horseradish peroxidase–conjugated goat antibody to rabbit immunoglobulin (Cappel). Micromolar concentrations of ionomycin are indicated at top. (**C**) AODH 7.1 cells were treated with 2 μM ionomycin (●), 2 μM ionomycin in the presence of 5 mM EGTA (▲), 5 mM EGTA alone (△), or left untreated (○). All of the experiments have been performed at least three times with similar results.

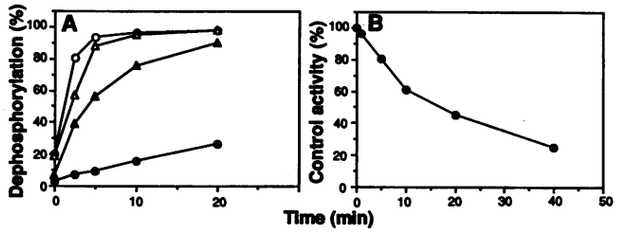

Fig. 2. Ionomycin treatment of mouse thymocytes and spleen cells. (**A**) Freshly isolated BALB/c thymocytes (circles) or spleen cells (triangles) were treated with 2 μM ionomycin for 20 min at 37°C (closed symbols) or left untreated (open symbols) and the CD45 assayed for PTPase activity as in Fig. 1. (**B**) Thymocytes were treated for various times with 2 μM ionomycin and then lysed; CD45 was then immunoprecipitated, and thymocytes were assayed for PTPase activity as in Fig. 1.

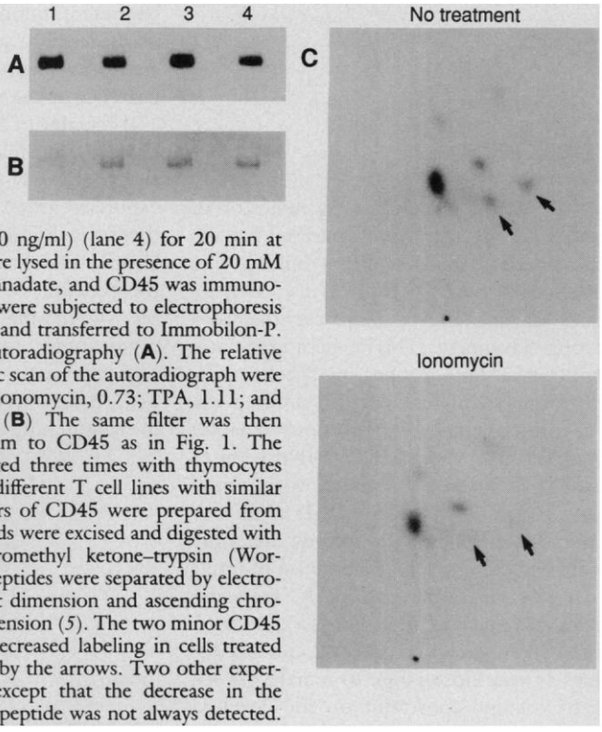

Fig. 3. Ionomycin treatment of mouse thymocytes decreases the level of phosphorylation of CD45. Thymocytes were labeled for 5 hours with $^{32}P_i$. The cells were then either untreated (lane 1) or treated with 2 μM ionomycin (lane 2), TPA (10 ng/ml) (lane 3), or 2 μM ionomycin and TPA(10 ng/ml) (lane 4) for 20 min at 37°C as in Fig. 1. The cells were lysed in the presence of 20 mM EDTA and 0.5 mM sodium vanadate, and CD45 was immunoprecipitated. The precipitates were subjected to electrophoresis on a 7.5% polyacrylamide gel and transferred to Immobilon-P. The filter was analyzed by autoradiography (**A**). The relative intensities from a densitometric scan of the autoradiograph were as follows: no treatment, 1.0; ionomycin, 0.73; TPA, 1.11; and TPA and ionomycin, 0.64. (**B**) The same filter was then immunoblotted with antiserum to CD45 as in Fig. 1. The experiments have been repeated three times with thymocytes and various times with four different T cell lines with similar results. (**C**) Immobilin-P filters of CD45 were prepared from thymocytes as in (A). The bands were excised and digested with *N*-tosyl-L-phenylalanine chloromethyl ketone–trypsin (Worthington Biochemical). The peptides were separated by electrophoresis at pH 8.9 in the first dimension and ascending chromatography in the second dimension (*5*). The two minor CD45 phosphopeptides that show decreased labeling in cells treated with ionomycin are indicated by the arrows. Two other experiments gave similar results except that the decrease in the labeling of the major phosphopeptide was not always detected.

tivity can be regulated in vivo and suggest that dephosphorylation of specific serine residues is involved. However, neither the sites of phosphorylation nor the enzymes involved have been identified. Several calmodulin-dependent protein Ser-Thr kinases could indirectly modulate the level of phosphorylation of CD45 by acting on other kinases or phosphatases (*19, 20*). Alternatively, protein Ser-Thr phosphatases of the PP-2B type have an absolute requirement for Ca^{2+}; activation of such a phosphatase after ionomycin treatment could directly lead to dephosphorylation of CD45 (*21*). A Ca^{2+}-calmodulin dependent phosphatase associates with the membrane of Jurkat cells and of normal human T lymphoblasts (*22*) and could be involved in regulating CD45 PTPase activity. It will be important for investigators to determine the specific serine residues in CD45 that are dephosphorylated after treatment with ionomycin and to identify the enzymes involved.

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