To assess the transfer of anthropogenic CO_2 to the oceans as a whole, it is necessary to consider the extent to which ocean surface increases in TCO_2 will affect the exchange of carbon with deeper waters. In both the advective and diffusive approaches to modeling vertical ocean mixing, the flux of anthropogenic CO_2 into the deep ocean is directly proportional to the amount of anthropogenic CO_2 in the mixed layer (*20, 21*). Thus, a 1 percent error in the mixed layer TCO_2 increase will generate a 1 percent error in the deep ocean TCO_2 increase. In comparison, advective and diffusive ocean mixing parameters are presently associated with uncertainties of at least 50 percent (*22*). These uncertainties are compounded by the unknown but nonnegligible involvement of horizontal transport processes. Present uncertainties in the nature of ocean mixing therefore completely overshadow uncertainties that might be generated by errors in the ocean surface TCO_2 increase.

Uncertainties in terrestrial carbon fluxes also contribute significantly to the monumental difficulty of modeling the global CO_2 system. For example, the range of estimates for the flux of CO_2 into the atmosphere from deforestation (*23*) exceeds the magnitude of the present annual flux from combustion of fossil fuels and is far greater than the range of errors in the CO_2 balance that might be attributed to homogeneous buffer factor measurements and calculations.

The global CO_2 problem requires an urgent effort to fill fundamental gaps in our knowledge of the global carbon system. In attempting to establish research priorities that will provide the quickest answers to the most important questions, the scientific community must carefully ascertain what we know as well as what we do not know. The homogeneous equilibrium behavior of dissolved inorganic carbon in surface seawater seems to be one of the best-known aspects of the present and future global CO_2 budget.

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Phospholipid Methylation Unmasks Cryptic β-Adrenergic Receptors in Rat Reticulocytes

Abstract. *The effect of phospholipid methylation on the number of β-adrenergic receptor binding sites was examined in rat reticulocyte membranes. Stimulation of phosphatidylcholine synthesis by the introduction of the methyl donor* S-*adenosyl-*L-*methionine into reticulocyte ghosts increased the number of β-adrenergic receptor binding sites. The appearance of β-adrenergic binding sites was dependent on the formation of phosphatidylcholine by the enzyme that converts phosphatidyl-*N-*monomethylethanolamine to phosphatidylcholine, but not on the synthesis of phosphatidyl-*N-*monomethylethanolamine from phosphatidylethanolamine. Both the synthesis of phosphatidylcholine and the unmasking of cryptic receptors were time and temperature dependent and did not occur in the presence of the methyl transferase inhibitor,* S-*adenosyl-*L-*homocysteine.*

Two membrane-bound enzymes are present in adrenal medulla, erythrocytes, and reticulocytes that synthesize phosphatidylcholine by successive *N*-terminal methylations of phosphatidylethanolamine (*1*). Both the enzymes and their products are asymmetrically distributed in the membrane. Methyltransferase I is located on the cytoplasmic side of the membrane and converts phosphatidylethanolamine to phosphatidyl-*N*-monomethylethanolamine. This enzyme has a high affinity (Michaelis constant, K_m, = 1 μM) for the methyl donor, *S*-adenosyl-L-methionine, and requires Mg^{2+}. Methyltransferase II, located on the external side of the membrane, adds two methyl groups to phosphatidyl-*N*-monomethylethanolamine to form phosphatidylcholine. This enzyme has a low affinity (K_m = 100 μM) for *S*-adenosyl-L-methionine and does not require Mg^{2+}. By these enzymatic reactions phosphatidylethanolamine, on the cytoplasmic side of the reticulocyte membrane, is methylated to phosphatidylcholine and rapidly translocated to the external side of the membrane (*1*).

Rat reticulocyte membranes contain relatively large numbers of β-adrenergic receptors (*2*). Stimulation of β-adrenergic receptors increases the activity of both methylating enzymes, particularly methyltransferase II (*3*). The elevation of phospholipid methylation depends on

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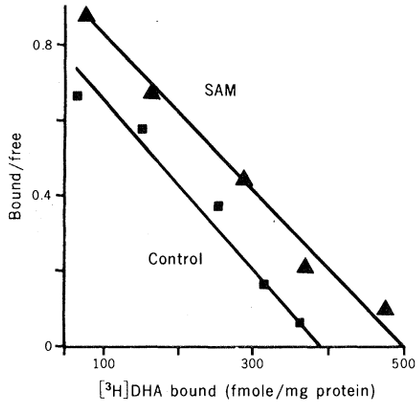
the number of available β-adrenergic receptors, but not on activation of adenylate cyclase. Stimulation of β-adrenergic receptors increases the methylation of both phospholipid substrates, and also enhances the rate of translocation of phospholipids from the cytoplasmic side of the membrane to the outside (*3*). Accumulation of phosphatidyl-*N*-monomethylethanolamine, the product of methyltransferase I, increases both membrane fluidity (*4*) and coupling between β-adrenergic receptors and adenylate cyclase, presumably by facilitating lateral movement of the receptor within the membrane (*3*). In view of the relation between membrane phospholipids and β-adrenergic receptors, we examined the effect of changes in phospholipid methylation on the number of β-adrenergic receptor sites. We report that stimulation of phosphatidylcholine synthesis increases the number of available β-adrenergic receptors in rat reticulocyte membranes.



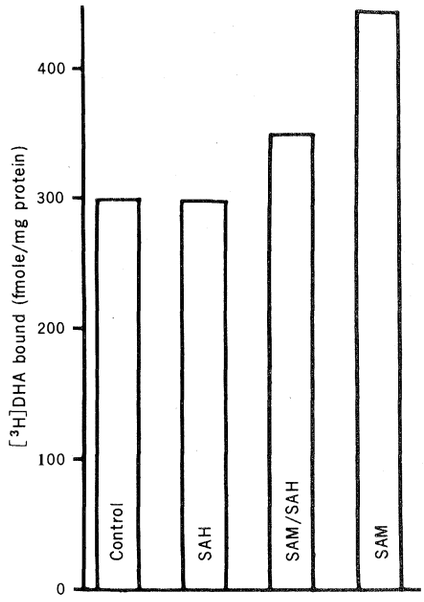


Fig. 1 (left). Scatchard analyses of [^{3}H]dihydroalprenolol binding to reticulocyte ghosts. Reticulocytes were generated by injecting rats with 0.5 ml of phenylhydrazine (15 mg/ml) on three consecutive days, and were obtained by cardiac puncture on day 7 (*2*). Ghosts were prepared by washing heparinized blood with 0.9 percent NaCl and subjecting the cells to hypotonic lysis in $MgCl_2$ as described (*1*). *S*-Adenosyl-L-methionine (200 μ*M*) was introduced into reticulocyte ghosts by incubating them overnight at 4°C in 50 m*M* tris-glycylglycine with 5 m*M* $MgCl_2$. The resealed ghosts were then washed by centrifugation and resuspended in 100 m*M* tris-glycylglycine with 10 m*M* $MgCl_2$. Reticulocyte ghosts prepared with (▲) and without (■) *S*-adenosyl-L-methionine (*SAM*) were then incubated for 60 minutes at 38°C. Specific [^{3}H]dihydroalprenolol binding was determined in varying concentrations (0.2 to 20 n*M*) of [^{3}H]dihydroalprenolol, in the presence and absence of 5×10^{-6} *M d,l*-propranolol as described (*2*). The data were plotted as the ratio of bound over free [^{3}H]dihydroalprenolol against [^{3}H]dihydroalprenolol bound. The lines were plotted by linear regression analyses. For each line the K_m was calculated as the negative inverse of the slope, and the number of binding sites was computed from the intersection of the line with the abscissa. Each point represents the mean of duplicate determinations in one experiment. The experiment was performed twice. The number of binding sites for control ghosts was 383 fmole per milligram of protein, compared to 517 for ghosts containing *S*-adenosyl-L-methionine. The K_m for control ghosts was 2.3 n*M*, and for ghosts containing *S*-adenosyl-L-methionine, 2.1 n*M*. Fig. 2 (right). Effect of the methyltransferase inhibitor *S*-adenosyl-L-homocysteine on the number of β-adrenergic receptor binding sites. Reticulocyte ghosts were prepared as described in Fig. 1, containing 200 μ*M S*-adenosyl-L-homocysteine (*SAH*); 200 μ*M S*-adenosyl-L-methionine and 200 μ*M S*-adenosyl-L-homocysteine (*SAM/SAH*); and 200 μ*M S*-adenosyl-L-methionine (*SAM*). They were then incubated for 60 minutes at 38°C, and the number of β-adrenergic receptor binding sites was determined by using saturating concentrations (20 n*M*) of [^{3}H]dihydroalprenolol. Results shown are the mean of duplicate determinations. The experiment was performed twice.

To demonstrate the appearance of new β-adrenergic receptor binding sites, we incubated reticulocyte ghosts containing 200 μ*M S*-adenosyl-L-methionine for up to 60 minutes at 38°C. The ghosts were then assayed for the number of β-adrenergic receptor sites that bind the β-adrenergic receptor ligand [^{3}H]dihydroalprenolol. In a typical experiment the number of binding sites increased linearly over 60 minutes from 417 to 513 fmole of [^{3}H]dihydroalprenolol bound per milligram of protein. In 11 separate experiments the number of β-adrenergic receptor sites after 60 minutes of incubation increased from 389 ± 23 to 522 ± 35 fmole of [^{3}H]dihydroalprenolol bound per milligram of protein ($P < .01$ by Student's *t*-test). No change in β-adrenergic receptor number occurred at 0°C, and no significant phospholipid methylation could be detected at this temperature. The appearance of newly available β-adrenergic receptor sites depended on both methylation and the integrity of the ghost membrane. Leaky ghosts prepared in hypotonic buffer, or membrane fragments, could methylate phospholipids but did not change the number of receptor binding sites. Leaky ghosts lose the asymmetry of membrane fluidity (*5*). The formation of newly synthesized phospholipid is therefore not sufficient to increase the binding of dihydroalprenolol. Reticulocytes incubated with and with-

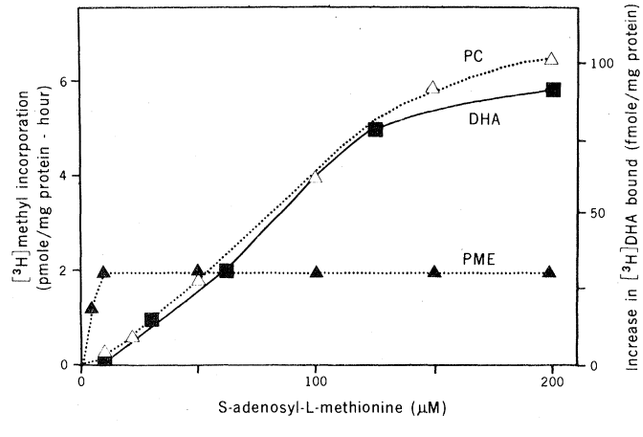


Fig. 3. Effect of *S*-adenosyl-L-methionine concentration on the number of β-adrenergic binding sites and phospholipid synthesis. Reticulocyte ghosts prepared as described in Fig. 1, with varying concentrations of *S*-adenosyl-L-methionine (0 to 200 μ*M*) were incubated for 60 minutes at 38°C and specific [^{3}H]dihydroalprenolol binding was determined (■). For identification of phospholipid product, reticulocyte ghosts were prepared containing *S*-adenosyl-[methyl-^{3}H]-L-methionine and unlabeled *S*-adenosyl-L-methionine (0 to 100 μ*M*), and incubated for 60 minutes at 38°C. After incubation, the phospholipids were extracted in a mixture of chloroform, methanol, and 2*N* HCl (1200 : 600 : 100, by volume), washed twice with 0.1*N* KCl in 50 percent methanol, and evaporated to dryness (*1*). The [^{3}H]methylated phospholipids were resuspended in chloroform methanol and cochromatographed with authentic, unlabeled, phosphatidylcholine (*PC*) and phosphatidyl-*N*-monomethylethanolamine (*PME*) on silica gel G plates in a solvent system of *n*-propyl alcohol, propionic acid, chloroform, and water (3 : 2 : 2 : 1, by volume). The [^{3}H]methylated products phosphatidylcholine (△), and phosphatidyl-*N*-monomethylethanolamine (▲) were then quantified by liquid scintillation spectroscopy.

out *S*-adenosyl-L-methionine demonstrated saturable binding of [^{3}H]dihydroalprenolol. Scatchard analysis of saturation curves on ghosts previously incubated with *S*-adenosyl-L-methionine revealed an increase in the number of binding sites, with no change in their affinity for [^{3}H]dihydroalprenolol (Fig. 1). *S*-Adenosyl-L-homocysteine inhibits the methyltransferases (*1*). Introduction of 200 μM *S*-adenosyl-L-homocysteine and 200 μM *S*-adenosyl-L-methionine into reticulocyte ghosts inhibited both the formation of phosphatidylcholine and the appearance of new β-adrenergic receptor sites approximately 55 percent (Fig. 2).

To determine whether one or both methyltransferases were responsible for the increase in the number of β-adrenergic receptors, we incubated reticulocytes with varying concentrations of *S*-adenosyl-L-methionine. After 60 minutes of incubation, we measured phosphatidyl-*N*-monomethylethanolamine, phosphatidylcholine, and the number of β-adrenergic receptor binding sites. The number of [^{3}H]dihydroalprenolol binding sites increased with the synthesis of phosphatidylcholine, but not with the synthesis of phosphatidyl-*N*-monomethylethanolamine (Fig. 3). These observations indicated that the increase in the number of β-adrenergic receptors that were made available for binding was related to the increased synthesis of phosphatidylcholine.

After incubation of reticulocytes for 60 minutes at 38°C, adenosine 3′,5′-monophosphate (cyclic AMP) accumulation with isoproterenol or sodium fluoride was reduced tenfold compared to unincubated controls. Thus the incubation conditions needed to increase the number of β-adrenergic receptors made the measurement of coupling between the unmasked receptors and adenylate cyclase technically infeasible.

The mechanism by which phospholipid methylation uncovers hidden receptors is unknown. Since de novo synthesis of new receptors is not possible in a purified membrane preparation, the appearance of new receptor sites may involve changes in charge or microenvironment of the membrane surrounding the receptor binding site. Depending on the physiological conditions, it has been shown that the number of β-adrenergic receptor sites can be rapidly increased or decreased in the rat pineal gland (*6*) and frog erythrocyte (*7*) even after inhibition of protein synthesis. Previous studies have shown that hydrolysis of membrane phospholipids reduces the number of available β-adrenergic receptors (*8*). The activities of certain membrane-bound enzymes, such as Ca^{2+}- and Mg^{2+}-dependent adenosinetriphosphatase and Na^+- and K^+-dependent adenosinetriphosphatase have been found to depend on the presence of phospholipids (*9*) and may also be influenced by phospholipid methylation.

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Homoeologous Heterozygosity and Recombination in the Fern *Pteridium aquilinum*

Abstract. *The bracken fern,* Pteridium aquilinum, *which can form completely homozygous zygotes in a single generation of self-fertilization, has a genetic system that allows the storage and release of genetic variability in spite of this homozygosity. Analysis of the distribution of electrophoretically demonstrable genetic markers demonstrates that this system is based on recombination between duplicated, unlinked loci.*

Among vascular plants, homosporous pteridophytes appear to be the only ones whose spores may give rise to gametophytes, each of which may form both male and female gametes. Because the gametes arise through mitotic cell division, all those produced by an individual gametophyte have identical genotypes. Consequently, self-fertilization of a gametophyte (intragametophytic selfing) produces zygotes with completely homozygous genotypes (*1*). It has been suggested (*2*) that, in adaptive relationship to this aspect of their life cycle, homosporous ferns have evolved a genetic system which permits the expression of genetic variability in the meiotic products (spores) formed by sporophytes that are homozygous in the conventional sense. In these ferns, which are polyploid (*1*), genetic variation is believed to be stored in the form of duplicated, unlinked loci (homoeologous heterozygosity), and released through the process of homoeologous pairing of chromosomes at meiosis. Thus, sporophytes produced through intragametophytic selfing are homozygous at the homologous-gene level but can carry homoeologous heterozygosity (*3*).

Evidence supporting this hypothesis has been of three kinds: the expression of chromosomal aberrations in homozygous spore-mother cells formed by sexual and apogamous species (*4*), the correspondence of segregation ratios of morphological marker genes to those predicted by models of tetrasomic inheritance (*5*), and the segregation of gametophytic mutants (*6*). But none of these lines of evidence unequivocally demonstrates homoeologous heterozygosity or recombination. We now present evidence derived from the demonstration of heterozygosity and recombination at polymorphic structural genes in homozygous sporophytes, and the inheritance of these genetic markers in families of the bracken fern, *Pteridium aquilinum*.

Pteridium aquilinum is a diverse species found throughout the world in temperate and tropical areas. Taxonomically it is polytypic, with two subspecies further subdivided into 12 varieties (*7*). Although much subspecific taxonomic variability is present, most taxa have the same chromosome number, $n = 52$, $2n = 104$ (*8*). Homozygous sporophytes representing both subspecies of *P. aquilinum* were grown from collections of spores made in Florida, Mexico, Colombia, and England (*9*). To ensure a

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