Membrane/Permease/ATPase/Transport

2118 PHARMACOLOGY

COMPARATIVE EFFECTS OF SUBSTITUTED PHENOTHIAZINES AND THEIR FREE RADICALS ON BRAIN (Na^++K^+)-ATPase ACTIVITY. R. H. Quintt* and T. Akera* (SPON: K. W. Cochran). Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823.

The effects of various phenothiazine tranquilizers and their free radicals on rat brain microsomal (Na^++K^+)-ATPase activity were compared. When the formation of free radical was carefully avoided, all phenothiazine derivative studied, namely promazine, chlorpromazine, thioridazine, trifluoperazine, perphenazine and trifluoperazine, had minimal inhibitory effects. The conditions which would produce free radicals, 254 nm irradiation of the drug-enzyme mixture or preincubation of the mixture in the presence of H_2O_2 and peroxidase, markedly enhanced the inhibition. The amounts of phenothiazine derivatives oxidized by a timed 254 nm irradiation varied widely among different derivatives. With thioridazine, the oxidation of approximately 0.5 nmoles/ml of the mixture produces a 50% inhibition of enzyme activity whereas with promazine and chlorpromazine, the oxidation of more than 3 nmoles/ml was required to produce comparable inhibition. A similar variation in inhibitory effect was also obtained when the free radicals were generated by the H_2O_2-peroxidase system. Under these conditions the inhibitory effects of phenothiazine derivatives on (Na^++K^+)-ATPase depends on the free radical. The free radicals of various derivatives may have different affinities for the enzyme. (Supported by NIMH-12783-06.)

2119 PHARMACOLOGY

CHLORINATED BIPHENYL INTERACTION WITH BEEF BRAIN (Na^++K^+)ATPase. Wm. E. Wilson* and C. W. Sharp*. (SPON: H. S. Posner). H.I.F.R.S., N.C.N.C., Research Triangle Park, N.C. 27709.

Inhibition of beef brain (Na^++K^+)ATPase by chlorinated biphenyls (CBs) has been compared with results obtained previously with several classes of the chlorinated hydrocarbon pesticides (Fed. Proc., 30(2): 673, 1971). The (Na^++K^+)ATPase (40 ug protein/ml) is inhibited 50% upon exposure to 8 to 14 ppm of Aroclor 1221 (a commercial mixture, primarily, of mono- and dichlorinated biphenyls), Aroclor 1254 (a mixture of biphenyls substituted with 3 or more chlorines) or DDT. Several subfractions of Aroclor 1221 obtained by distillation, 2-chlorobiphenyl and 3-chlorobiphenyl were about as inhibitory as Aroclor 1221, whereas, biphenyl, 4-chlorobiphenyl and 4,4'-dichlorobiphenyl were less inhibitory. Upon addition to the aqueous incubation medium, the ethanolic solution of each CB or CB mixture underwent a change of turbidity over a period of seconds to hours. Each of the CBs was inhibitory upon addition of ethanolic solutions of these compounds to the incubation medium; however, only Aroclor 1254 yielded significant inhibition when exposure to the (Na^++K^+)ATPase was as a previously prepared dispersion of the oil in water. As in the case of DDT inhibition, phosphatidyl serine protected beef brain (Na^++K^+)ATPase from inhibition by CBs. Thus, beef brain (Na^++K^+)ATPase inhibition by the CBs simulates several aspects of enzyme responsiveness to chlorinated hydrocarbon pesticides.

2120 PHARMACOLOGY

KINETICS OF INHIBITION OF (Na^++K^+)-ATPase BY DDT AND ALLETHRIN. J. F. Schneider* (SPON: E.B. Truitt). Food and Biol. Sci.Div. Battelle-Columbus Laboratories, Columbus, Ohio 43201

The (Na^++K^+)-ATPases in swine erythrocyte membrane fragments and rat brain microsomes are inhibited by DDT and allethrin. The kinetics of inhibition by these compounds are similar in both preparations. Both of these compounds interact competitively with Na^+ in the case of the enzyme from erythrocytes. The concentration of DDT to cause one-half of maximal inhibition is about 4 umoles/l for the brain enzyme and 30 umoles/l for the erythrocyte preparation. The enzyme from brain shows mixed inhibition kinetics with respect to Na^+ in the presence of K^+ concentrations less than 40 mM. At higher concentrations of K^+ the kinetics are competitive with Na^+. When K^+ is varied but held below 1 mM, inhibition is noncompetitive with K^+. Inhibition is increased several fold, however, by increasing K^+ concentrations from 10 to 100 mM. The apparent competitive interaction of these compounds with Na^+ tends to support earlier suggestions that DDT specifically blocks Na^+ sites or receptors in cell membranes. The effects of DDT and allethrin together are not additive, suggesting a common site of inhibition. The structure of allethrin does not resemble that of DDT, but they have similar effects on electrical activity of nerves. The apparent blocking of Na^+ activation and the common mode of inhibition of the (Na^++K^+)-ATPase by DDT and allethrin suggests that inhibition of this enzyme may be analogous to effects on nerve function. (Supported by Battelle Institute).

2121 PHARMACOLOGY

MECHANISM OF INHIBITORY EFFECTS OF CARDIAC GLYCOSIDES ON RED CELL METABOLISM. P. Okonkwo* and A. Askari. Department of Pharmacology, Cornell Univ. Med. Col. New York, N. Y. 10021.

Cardiac glycosides (CG) affect the metabolism of several tissues. In red cells they cause partial inhibition of lactate production. Since CG do not affect the enzymes of glycolytic pathway, it has been suggested (Hoffman and Parker, J. Gen. Physiol. 50, 893, 1967) that CG exert their metabolic effect by inhibiting the pump-ATPase, and reducing the size of an ADP pool that is the preferred substrate for phosphoglycerate kinase(PGK). To test this hypothesis, purified and crystalline PGK from human red cells was prepared by modification of existing methods. An antibody (AB) to PGK was made. AB inhibited PGK activities of hemolysed red cells, ghost-free hemolysates, and broken ghosts. Effects of AB and CG on lactate production from fructose-1,6-diphosphate were tested. In ghost-free hemolysates lactate production was inhibited by AB but not by CG. In the presence of ghosts there are two components of lactate production: CG-sensitive and CG-insensitive. When increasing concentrations of AB were added, CG-insensitive component disappeared first. With proper choice of AB concentration conditions could be obtained under which nearly all of lactate production became sensitive to CG. These results suggest that there is a pool of PGK that is primarily dependent on the substrate that is generated within the membrane. (Supported by USPHS Grants HE-10884 and 5 T01 GM00069).

2122 PHARMACOLOGY

INHIBITORY EFFECT OF DEHYDROTHIAZIDE ON SODIUM-POTASSIUM ACTIVATED ADENOSINE TRIPHOSPHATASE OF CHOROID PLEXUS IN CAT AND RABBIT. Chaim L. Heyman* (SPON: H. J. Uretsky). Department of Neurology, Beth Israel Hospital and Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts 02215.

Dexamethasone-21-phosphate (Dm-P), at a concentration of 10^{-4} M caused total inhibition of the sodium-potassium (Na^+K^+) activated portion of the adenosine triphosphatase (ATP'ase) activity, in-vitro, in choroid plexus of cats anesthetized with intravenous pentobarbital. Similar results were obtained using rabbit choroid plexus obtained commercially. A moderate degree of inhibition of the magnesium stimulated portion of ATP'ase activity was also observed. Dm-P induced inhibition was greater than that of ouabain, 10^{-4}M. Dm-P induced a greated inhibition than did hydrocortisone, cortisone and desoxycorticosterone. It has been noted in the past that Dm-P causes a reduction in spinal fluid formation. It has been suggested that this effect may be mediated by influencing the secretory mechanism of spinal fluid formation. A similar effect has been observed with ouabain, a known inhibitor of Na-K ATPase. It is suggested that reduction in spinal fluid formation seen with Dm-P may be mediated by its effect on Na-K ATP'ase activity. (Supported, in part, by a GRS grant from the Beth Israel Hospital and NIH-NS 05172.)

2123 PHARMACOLOGY

POTASSIUM-INDUCED CONFORMATIONAL TRANSITION OF THE OUABAIN-(Na^++K^+)-ATPase COMPLEX: FLUORESCENT PROBE STUDIES. T. Akera*, S. I. Baskin*, and T. Tobin* (SPON: H. H. Swain) Department of Pharmacology, Michigan State University, East Lansing, Michigan 48823.

We have proposed that K^+ induces a conformational change in the ouabain-(Na^++K^+)-ATPase complex prepared by Na^+-ATP-dependent binding such that the binding sites for ouabain become less accessible (Akera and Brody, J. Pharmacol. Exp. Therap. 176:545, 1971). To obtain more direct evidence, the effect of K^+ on the fluorescence of 1-anilino-8-naphthalene sulfonate (ANS) bound to the rat brain enzyme preparation was studied. In the absence of the enzyme protein, ANS had minimal fluorescence. The addition of ANS to a mixture containing ouabain-enzyme complex, Na^+, Mg^{++} and ATP, resulted in the typical fluorescence of hydrophobically bound ANS with peak excitation and emission wavelengths at 385 and 480 nm. The addition of K^+ to this mixture produced a slight decrease in fluorescence intensity. The addition of K^+ prior to ANS resulted in a significantly lower fluorescence intensity. The intensity was lower and the effects of K^+ were minimal or absent when the ouabain was omitted from the mixture or the complex was prepared by using substrates, such as acetyl phosphate or PI instead of ATP. K^+ alters the conformation of the ATP-dependent (Na^++K^+)-ATPase complex so that hydrophobic binding by ANS is reduced. (Supported by NIMH-12783-06 and by the Mich. Heart Assoc.)

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