RELATIONSHIPS BETWEEN DRUG-INDUCED PERTURBATION OF Na^+/K^+-ATPASE ACTIVITY AND SYNAPTIC PLASMA MEMBRANE STRUCTURE

by

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ABSTRACT

Alterations in rat SPM bilayer structure and Na^+/K^+-ATPase activity were quantitated and correlated for drugs that are both structurally related and exhibit similar toxicities but belong to diverse pharmacological classes (tricyclic antidepressants, phenothiazines and antihistamines). The objectives of this study were to search for common mechanisms at the molecular level that may underlie their toxicology and to identify predictive techniques based on potential structure-activity relationships. This study shows that Na^+/K^+-ATPase IC_{50} values decrease linearly with increasing octanol/water partition coefficients for a series of structurally similar, dimethylamine-containing drugs (i.e. chlorpromazine, amitriptyline, imipramine, doxepin and diphenhydramine), emphasizing the importance of hydrophobicity in inhibition of this enzyme. However, the N-demethylated forms of amitriptyline and imipramine (nortriptyline and desipramine, respectively) are over a log unit less hydrophobic than their parent drugs but yet are more potent inhibitors of Na^+/K^+-ATPase. In order to examine this inconsistency, bilayer structure was evaluated by determining both the binding characteristics of the fluorescent biomembrane probe ANS to SPMs and the changes in SPM-bound DPH anisotropy in the absence and presence of these drugs. The dissociation constant (K_{app}) of ANS is invariant indicating no drug-induced change in the strength of ANS binding to SPMs. However, the increase in the limiting fluorescence intensity of ANS (F_∞) along with the unchanged λ_{max} indicates that these drugs perturb the membrane surface as to increase the number but not the polarity of ANS binding sites, apparently by charge

shielding the anionic portion of phospholipids. Not only does each drug inhibit Na^+/K^+-ATPase activity and increase the F_∞ of ANS over the same pharmacological/toxicological concentration range, but more importantly, there is a linear correlation between the concentrations of drugs necessary to increase the F_∞ of ANS by 40% and the IC_{50} values for all drugs studied with full compensation for the N-demethylated drugs. This correlation implies that drug-induced increases in SPM-bound ANS fluorescence are a better predictor of Na^+/K^+-ATPase inhibition than the octanol/water partition coefficient. These drugs inhibit K^+-*p*NPPase activity (the reaction sequence catalyzing K^+-dependent dephosphorylation in the Na^+/K^+-ATPase reaction cycle) with the same potency as Na^+/K^+-ATPase activity indicating that inhibition may involve interaction of the drugs near the extracellular K^+ transport site. The effects of chlorpromazine, diphenhydramine and dimethylaminopropyl chloride on K^+-*p*NPPase kinetics were quantitated by Lineweaver-Burk plots. Inhibition of K^+-activation progresses from noncompetitive by chlorpromazine through mixed by diphenhydramine to competitive by dimethylaminopropyl chloride. This progression, together with molecular modeling and diffraction studies (by others), is consistent with a mechanism by which drugs of different hydrophobicity project into the membrane bilayer to varying depths causing a corresponding alteration in the position of the drug's amino group with respect to the K^+ binding site(s). In contrast to the ANS data, drug-induced decreases in DPH anisotropy do not correlate with Na^+/K^+-ATPase inhibition, and drug N-demethylation enhances inhibition without altering anisotropy. Taken together, these data indicate that drug-induced changes in bulk fluidity do not predominantly influence

Na^+/K^+-ATPase activity, but rather that the crucial factor is electrostatic interactions at the membrane surface between the protonated amino group of the drug and anionic groups on the enzyme and/or phospholipids near the K^+ binding sites. Given that these drugs have similar toxicological activities and that changes in biomembrane function and structure can mediate deleterious effects, it is suggested that at some level the overlapping toxicities of these drugs are due to their similar mechanisms of membrane surface perturbation causing Na^+/K^+-ATPase inhibition or changes in other membrane-bound proteins.

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