KINETIC ANALYSIS OF OUABAIN-K^+ AND Na^+ INTERACTION ON A Na^+,K^+-DEPENDENT ADENOSINETRIPHOSPHATASE FROM CARDIAC TISSUE*

Hideo Matsui and Arnold Schwartz

Department of Pharmacology

Baylor University College of Medicine, Houston, Texas

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Antagonism by K^+ of ouabain inhibition of Na^+,K^+-dependent adenosinetriphosphatases (Na^+,K^+-ATPase), reported earlier by Dunham and Glynn (1961), Post and Albright (1961) and Auditore (1964), suggests that ouabain inhibits the enzyme activity by a displacement of K^+ from a K^+-binding site (Skou, 1964). On the other hand, Schatzmann (1965) observed a non-competitive interaction between ouabain and K^+ or Na^+ on a red cell ghost ATPase system.

The kinetics of the Na^+,K^+-ATPase as well as studies of partial reactions of the enzyme system have not been extensively pursued due, in part, to difficulty in purifying the enzyme. The present report is concerned with the kinetics of ouabain inhibition of a highly specific Na^+,K^+-ATPase from cardiac tissue (Matsui and Schwartz, 1966). The non-competitive nature of ouabain-K^+ interaction is described, stressing the dependency of ouabain-induced inhibition upon the Na^+/K^+ ratio.

METHODS

Enzyme: The preparation of a highly specific Na^+,K^+-ATPase from

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calf heart has been recently described (Matsui and Schwartz, 1966). More than 95% of the total ATPase activity of the enzyme was Na^+,K^+-dependent and ouabain-sensitive; the specific activity varied from 20 to 30 μmoles Pi/mg protein/hr.

Assay of ATPase: The reaction mixture, in a total volume of 1 ml, contained 50 - 100 μg enzyme protein, 2 mM ATP (Tris-salt), 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetate, 50 mM Tris-HCl (pH 7.4), and various concentrations of NaCl and KCl in the presence or absence of ouabain. Incubation was carried out at 37° for 10 min. After addition of ice-cold trichloroacetic acid, the liberated orthophosphate was determined by the method of Martin and Doty (1949). Protein was determined by the method of Lowry et al. (1951).

All results are expressed as Na^+,K^+-dependent ATPase activity which was calculated from the activity in the presence of Na^+ and K^+, subtracting the basal Mg^{2+}-dependent activity.

RESULTS AND DISCUSSION

At a constant level of Na^+ concentration, the percent inhibition produced by ouabain decreased with increasing concentrations of K^+; at a constant level of K^+, the percent inhibition by ouabain increased with increasing concentrations of Na^+. This finding, suggesting that Na^+ as well as K^+ affects ouabain inhibition, is in agreement with the work of Schatzmann (1965) and in contrast to that of Post and Albright (1961). The kinetics of the system with the above conditions, however, are difficult to interpret. This is probably due to the complicating presence of K^+ inhibition at the Na^+-site and of Na^+ inhibition at the K^+-site (Green and Taylor, 1964).

When the ratio of Na^+ and K^+ was kept constant in order to cancel the inhibitory interaction between Na^+ and K^+*, however, a

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double-reciprocal plot of ATPase activity and K^+ (i.e. Na^+), yielded a linear relationship except at very low concentrations of K^+ ($\leq$ 0.2 mM K^+). The inhibition by ouabain at a constant Na^+/K^+ ratio was analyzed by the method of Lineweaver and Burk (1934). As shown in Fig. 1, maximal velocities in the presence and absence of two concentrations of ouabain were dissimilar, and, moreover, each line met at the same intercept on the abscissa, indicating a non-competitive type of inhibition between ouabain and K^+ (or Na^+).

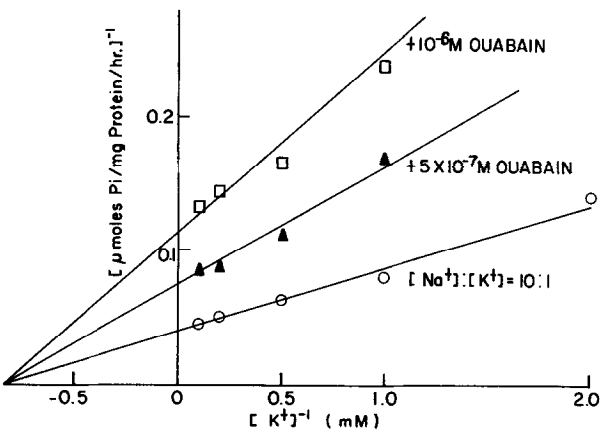


Fig. 1. Lineweaver-Burk analysis of ouabain inhibition at a constant Na^+/K^+ ratio. Na^+,K^+-ATPase activity was determined at various K^+ concentrations with a constant Na^+/K^+ ratio of 10 in the presence and absence of 5 x 10^{-7}M and 10^{-6}M ouabain; 1/v was plotted against $1/K^+$ ($=1/Na^+$ x 10).

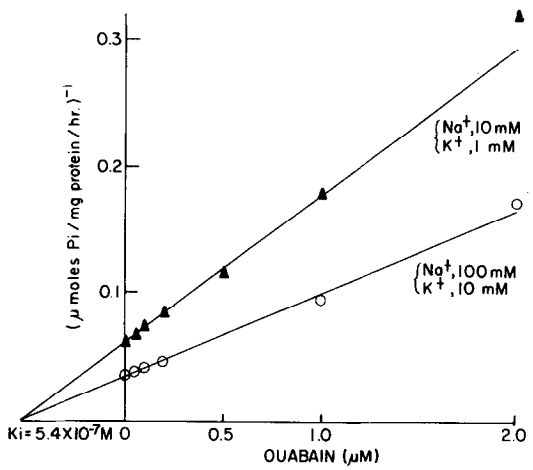


Fig. 2. Dixon plot of ouabain inhibition at a constant Na^+/K^+ ratio.

This non-competitive type of inhibition was also demonstrated by the method of Dixon (1953). The plot of 1/v against ouabain concentration yielded a linear relationship, and the lines corresponding to different K^+ concentrations with the same Na^+/K^+ ratio

met at the same intercept on the abscissa (Fig. 2). At a Na^+/K^+ ratio of 10, the Ki for ouabain obtained from the Dixon method (Fig. 2) was 5.3×10^{-7} M, and was the same as that calculated from the Lineweaver-Burk method (Fig. 1).

When the Dixon plot was constructed at different concentrations of K^+ and a constant level of Na^+ (Fig. 3a), the lines met approximately at one point, suggesting competitive kinetics. On the other hand, when Na^+ was varied while maintaining the K^+ concentration constant (Fig. 3b), the plots described neither competitive nor non-competitive kinetics (Dixon, 1953). However, since the ouabain-K^+ or Na^+ interaction is clearly of a non-competitive nature as shown in Figs. 1 and 2, there must be another interpretation for the results shown in Figs. 3a and b. It is proposed that the Ki for ouabain is dependent upon the Na^+/K^+ ratio. In Fig. 3, therefore, each line represents merely the different Ki's for ouabain, corresponding to a given ratio of Na^+/K^+ and the overall "family" of lines has no validity in determining the type of kinetics involved. Increasing the K^+ at a constant Na^+ concentration (i.e. decreasing the Na^+/K^+ ratio) caused an increase in the Ki (intercept on abscissa) (Fig. 3a), while increasing the Na^+ at a constant K^+ concentration (i.e. increasing the Na^+/K^+ ratio) decreased the Ki (Fig. 3b). The relationship between Ki and Na^+/K^+ ratio is graphically displayed in Figure 4 and may be mathematically expressed as:

$$Ki = 3.5 \times 10^{-6} \times (Na^+/K^+)^{-0.82}$$

This relationship obtains in the range of Na^+/K^+ ratio from 1 to 100.

From the results presented here, it is concluded that inhibition by ouabain is due neither to direct nor indirect displacement of K^+ at the K^+-site. Furthermore, the binding site of ouabain appears to be different from the K^+-site. Although ouabain inhibi-

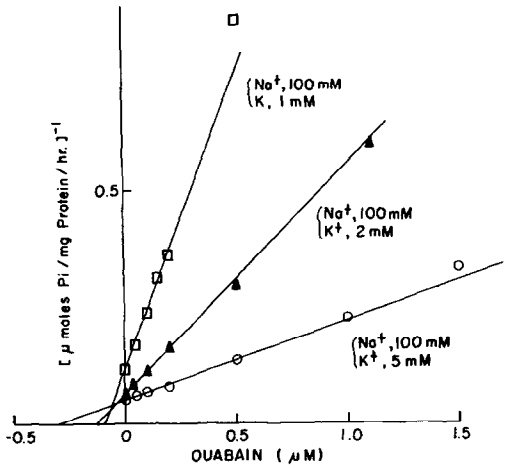


Fig. 3a. Dixon plot of ouabain inhibition at different Na^+/K^+ ratios. Varying K^+ with a constant Na^+ concentration.

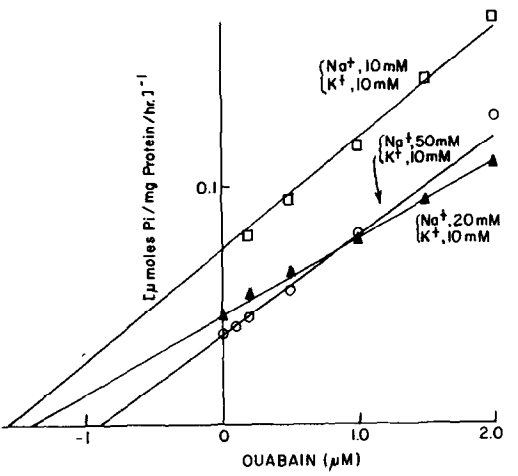
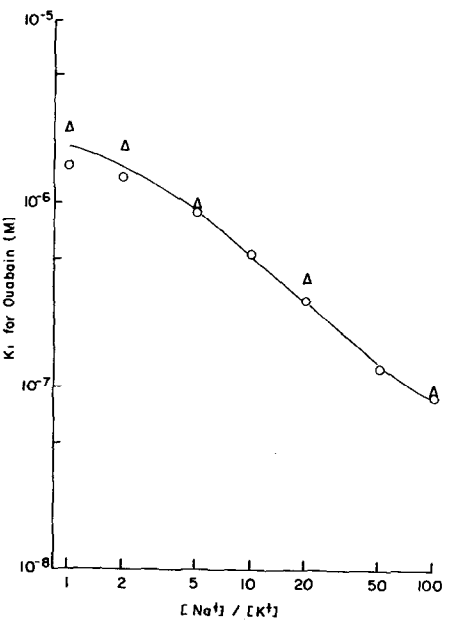


Fig. 3b. Dixon plot of ouabain inhibition at different Na^+/K^+ ratios. Varying Na^+ with a constant K^+ concentration.

Fig. 4. Relationship between Ki for ouabain and Na^+/K^+ ratio. Ki obtained was plotted against Na^+/K^+ ratios on a full logarithmic scale. O,Δ represent different enzyme preparations.



tion is not competitive with K^+, the dependency of the Ki for ouabain on the Na^+/K^+ ratio (Fig. 3a) could explain the antagonistic phenomenon between ouabain and K^+ reported earlier (Dunham and Glynn, 1961; Post and Albright, 1961; Auditore, 1964). These results are in accordance with Schatzmann's observations (1965), dealing with the interaction of Na^+ on ouabain inhibition. In this connection, inhibition by ouabain of a K^+-dependent p-nitrophenylphosphatase (Fujita et al., 1966) is of interest, for the Ki (3×10^{-6} M) in the absence of Na^+ is similar to the upper limit of the Ki on the Na^+,K^+-ATPase at low Na^+/K^+ ratios (Fig. 4).

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