DIFFERENT OLIGOMYCIN SENSITIVITIES OF THE Na^++K^+-ACTIVATED ADENOSINETRIPHOSPHATASE AND ITS PARTIAL REACTIONS.

A. Askari and D. Koyal

Department of Pharmacology, Cornell University Medical College
New York, New York 10021

Received June 13, 1968

The results of several studies, recently reviewed by Albers (1967), on the Na^++K^+-activated adenosinetriphosphatase (Na^+,K^+-ATPase) indicate that this enzyme activity consists of a Na^+-dependent phosphorylation of the enzyme by ATP, and a K^+-stimulated hydrolysis of the resulting acylphosphate (E∼P). The Na^+,K^+-ATPase preparations also catalyze the K^+-stimulated hydrolysis of several simple organic phosphates (Albers, 1967). In spite of some disparities between the properties of the two enzyme activities (e.g., Israel and Titus, 1967); since the phosphatase activities, like the Na^+,K^+-ATPase and K^+-stimulated E∼P breakdown, are inhibited by cardiac glycosides, the possibility of the identity of the phosphatase with the terminal hydrolase of ATPase has been considered. In a complex enzyme system such as the Na^+,K^+-ATPase, the determination of sites of actions of inhibitors often provides important clues to the mechanism of the overall reaction. In this report we present an account of our work on the effects of oligomycin on the Na^+,K^+-ATPase and the K^+-stimulated phosphatase. The results indicate that the K^+-stimulated phsophatase is indeed a partial reaction of the Na^+,K^+-ATPase. In conjunction with observations of others, our data also show that in addition to the Na^+-dependent E∼P formation there is a Na^+-dependent ATP binding to the enzyme system, and that the latter process has an activating effect on the K^+-stimulated hydrolytic step. Oligomycin either inhibits the Na^+-dependent ATP binding, or blocks the activating effect of bound Na^+-ATP on the hydrolysis reaction.

Na^+,K^+-ATPase from rat brain was prepared by the method of Skou (1962), and assayed as described before (Askari and Fratantoni, 1964). Acetylphospha-

Table 1. Effects of varying concentrations of oligomycin on the ATPase, AcPase, and NPPase activities. Substrate concentrations in a final volume of 2.5 ml were: ATP, 2mM; acetylphosphate, 4mM; p-nitrophenylphosphate, 4mM. $MgCl_2$ concentration was equal to substrate concentration in each case. Na^+ and K^+ were added at indicated concentrations as chlorides. The same amount of enzyme (0.1 mg protein) was used in each reaction. The enzyme activities are expressed as umoles of product formed (Pi for ATPase and p-nitrophenol for NPPase) or umoles of substrate (acetyphosphate) used per mg of protein per hr.

		Oligomycin (μg/ml)					
	Alkali cations	0	1	5	10	50	100
ATPase:	None	5.0	5.1	4.9	5.0	4.7	4.9
	Na^+, 10mM	6.0	6.3	6.1	5.9	5.9	5.8
	K^+, 1mM	5.1	5.1	4.8	4.9	5.3	5.0
	Na^+, 10mM + K^+, 1mM	33.0	21.0	14.1	10.8	10.1	10.3
AcPase:	None	3.1	3.1	2.9	2.9	3.2	3.1
	Na^+, 10mM	2.9	3.1	3.3	2.8	2.9	2.9
	K^+, 1mM	16.3	16.0	15.8	16.0	16.6	15.9
	Na^+, 10mM + K^+, 1mM	16.0	16.1	16.1	15.8	15.8	16.0
NPPase:	None	0.41	0.41	0.41	0.39	0.42	0.39
	Na^+, 10mM	0.40	0.41	0.38	0.38	0.38	0.38
	K^+, 1mM	2.45	2.50	2.51	2.48	2.43	2.46
	Na^+, 10mM + K^+, 1mM	2.46	2.46	2.46	2.44	2.44	2.44

tase activity (AcPase) was measured by the assay of acetyphosphate (Lipmann and Tuttle, 1945), and p-nitrophenylphosphatase (NPPase) was determined by the colorimetric assay of p-nitrophenol (Bessey et al, 1946). All substrates and oligomycin were obtained from Sigma Chemical Co.

Table 1 shows the effects of varying concentrations of oligomycin on the ATPase, AcPase, and NPPase activities of the same enzyme preparation. From the data, which confirm and extend the observations of Israel and Titus (1967), it is evident that (a) maximum inhibition of Na^+,K^+-ATPase (85%) is obtained at an oligomycin concentration of about 10μg/ml; (b) several concentrations of oligomycin which maximally inhibit the Na^+,K^+-ATPase do not affect the other phos-

phatase activities. Three possibilities which could explain these data are as follows: 1) K^+-stimulated phosphatase activities may have no relation to the enzyme which hydrolyzes E∼P. 2) The effect of oligomycin may be on the reactions leading to the formation of E∼P. 3) The oligomycin sensitive pathway of the Na^+,K^+-ATPase activity may not involve the formation of E∼P. The first possibility did not seem attractive to us since we had found (unpublished observations) four other classes of compounds which like cardiac glycosides inhibit both the Na^+,K^+-ATPase and the K^+-stimulated phosphatase. The second possibility has been ruled out directly (Whittam et al., 1964; Israel and Titus, 1967), and the third is not consistent with the data showing the sensitivity of the K^+-dependent E∼P breakdown to oligomycin (Whittam et al., 1964; Fahn et al., 1968). In view of these facts how can one reconcile the insensitivity of the K^+-stimulated phosphatase activities to oligomycin (Table 1) with the sensitivity of both the Na^+,K^+-ATPase and the K^+-dependent E∼P breakdown? It seemed that a difference between the reactions in which an oligomycin effect is obtained, and those which are not sensitive to oligomycin, is the presence of ATP and Na^+ in the former but not in the latter[1]. Accordingly, the effects of oligomycin on the K^+-stimulated phosphatases in the presence and absence of ATP and Na^+ were studied. Neither the presence of Na^+ alone (Table 1) nor that of ATP alone (Fig. 1) conferred any oligomycin sensitivity to the K^+-stimulated phosphatases[2]. In the presence of both Na^+ and ATP, however, results such as those shown in Fig. 1 were obtained. The data show that (a) in agreement with the observations of Nagai and Yoshida (1966) the simultaneous presence of Na^+ and low concentrations of ATP further activate the K^+-stimulated NPPase; (b)

1. Experimental conditions under which the oligomycin sensitivity of E∼P breakdown are demonstrated necessitate the presence of ATP and Na^+ (Whittam et al., 1964; Fahn et al., 1968).

2. The inhibitory effects of higher concentration of ATP on K^+-stimulated phosphatase (Fig. 1) have been observed by several other investigators. These effects are probably due to the highly charged ATP anion since many other anions have similar effects (our unpublished observations).

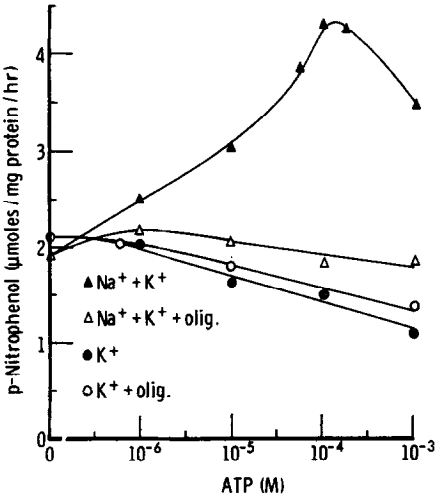


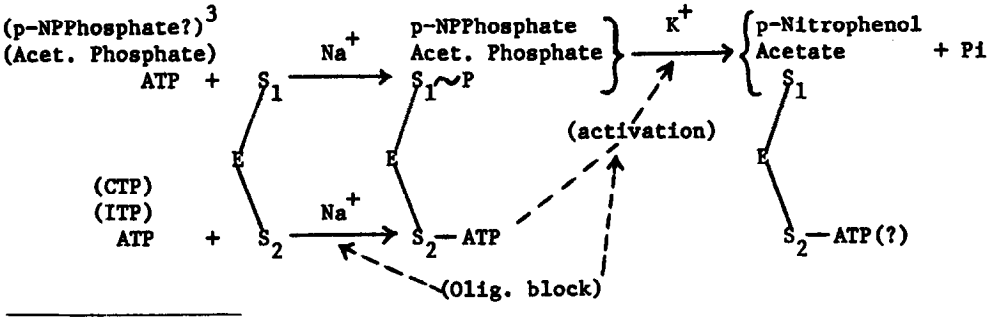
Fig. 1. Effects of oligomycin (25μg/ml) and varying concentrations of ATP on the K^+-stimulated NPPase activity in the presence and absence of Na^+. Experimental conditions were the same as those described for Table 1.

this extra activation is blocked by oligomycin. Since in the presence of ATP and Na^+ the formation of E∼P from ATP becomes possible, it may be concluded from the above data that it is the presence of E∼P that enhances the K^+-stimulated enzymic process which is responsible for the hydrolysis of E∼P and other phosphates. On this basis the oligomycin effect would have to be explained by a blocking action on this modifying effect of E∼P. There are certain experimental data, however, which do not fit such a hypothesis. First, it is known that E∼P can be formed in the presence of Na^+ not only from ATP but also from acetylphosphate (Bond et al., 1966; Israel and Titus, 1967). If it were the E∼P per se which had a modifying effect on the hydrolytic process, the presence of Na^+ alone should be sufficient to produce an oligomycin sensitive activation of the K^+-stimulated AcPase activity. Clearly this is not the case (Table 1). The second type of data which also argue against a modifying role of E∼P are presented in Table 2. Here it is shown that CTP and ITP, like ATP, activate the K^+-stimulated NPPase in the presence of Na^+; and that the activations by these nucleotides are also blocked by oligomycin. Considering

Table 2. Sensitivity of the nucleotide activated NPPase to oligomycin. Reaction conditions were the same as those described for Table 1.

	NPPase activity in the presence of:	
	no oligomycin	oligomycin (15ug/ml)
Na^+, 10mM + K^+, 1mM	2.21	2.22
" " " " + ATP, 0.5mM	3.90	2.20
" " " " + ITP, 0.5mM	3.40	2.19
" " " " + CTP, 0.5mM	3.35	2.20

that several studies have indicated the absence of formation of E~P from nucleotides other than ATP (e.g., Fahn et al., 1968), it is more plausible to assume that it is the presence of a nucleoside triphosphate and Na^+, rather than E~P, that creates a conformational change in the enzyme which is more favorable to the K^+-stimulated hydrolysis reaction. Furthermore, the data indicate that the binding site of nucleotides for this effect is different from the site which binds ATP leading to the E~P formation. The action of oligomycin may be envisioned to be either on the binding of Na^+-nucleotide to the modifying site, or on the process of formation of the "favored conformation". The working hypothesis is shown in the following scheme:



It should be pointed out that this hypothesis is also consistent with our obser-

3. The possibility of the formation of E~P from p-nitrophenylphosphate has not been investigated.

vation (Table 1) and those of others (Van Groningen and Slater, 1963; Israel and Titus, 1967) that the oligomycin inhibition of Na^+,K^+-ATPase is not complete.

From the above data and hypothesis, the question arises as to whether the nucleoside triphosphates which bind to the modifying site remain intact or not. It is interesting to note that on the basis of kinetic data Kanazawa *et al.* (1967) have suggested that the ATPase may be a "double headed" enzyme. The first site hydrolyzing ATP through the E∿P (the main pathway at 37°), and the second hydrolyzing ATP in the presence of Na^+ alone through a Michaelis complex. The possible identity of the second site proposed by these investigators with the modifying site indicated by our data remains to be determined.

Acknowledgment. This investigation was supported by U. S. Public Health Service Research Grant No. 10884 from the National Heart Institute.

References:

Albers, R.W., Ann. Rev. Biochem., 36, 727 (1967)
Askari, A. and Fratantoni, J.C., Biochim. Biophys. Acta, 92, 132 (1964)
Bessey, O.A., Lowry, O.H., and Brock, M.J., J. Biol. Chem., 164, 321 (1946)
Bond, G.H., Bader, H. and Post, R.L., Fed. Proc., 25, 567 (1966)
Fahn, S., Koval, G.J. and Albers, R.W., J. Biol. Chem. 243, 1993 (1968)
Israel, Y. and Titus, E., Biochim. Biophys. Acta, 139, 450 (1967)
Kanazawa, T., Saitō, M. and Tonomura, Y., J. Biochem., 61, 555 (1967)
Lipmann, F. and Tuttle, L.C., J. Biol. Chem., 159, 21 (1945)
Nagai, K. and Yoshida, H., Biochim. Biophys. Acta, 128, 410 (1966)
Skou, J.C., Biochim. Biophys. Acta, 58, 314 (1962)
Van Groningen, H.E.M. and Slater, E.C., Biochim. Biophys. Acta, 73, 527 (1963)
Whittam, R., Wheeler, K.P. and Blake, A., Nature, 203. 720 (1964)