IONOPHORETIC STIMULATION OF K^+-ATPase OF OXYNTIC CELL MICROSOMES

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SUMMARY The K^+-selective ionophores valinomycin, trinactin, nigericin, and gramicidin stimulated the K^+-ATPase of oxyntic cell microsomes as much as 5 fold. Gramicidin altered the K^+ activation kinetics: while the V_{max} greatly increased, the K_a for K^+ shifted only slightly. Activation also occurred with lyophilization, repeated freezing and thawing and nonionic detergents. The results are consistent with one population of membranes being freely permeable to K^+ (source of the K^+-dependent activity in absence of ionophore) and another population being impermeable to K^+. The K^+-ATPase in these latter vesicles would be activated by the appropriate ionophore or membrane disruptive procedure.

INTRODUCTION The acid secretory cell of the gastric mucosa, the oxyntic cell, contains a specialized membrane system where the molecular mechanism for HCl transport is thought to be located. This membrane system has been isolated as small tubules and vesicles from the microsomal fraction of oxyntic cell homogenates (1). The membranes have K^+-stimulated ATPase activity (1), carry out the K^+-dependent hydrolysis of p-nitrophenylphosphate (2, 3), and form a phosphorylated intermediate from (γ-^{32}P)ATP which is also sensitive to K^+ (4, 5). We now report on the stimulation of the K^+-ATPase by potassium ionophoretic substances.

METHODS The microsomal fraction of bullfrog oxyntic cell homogenates was isolated and purified by a slight modification of the method reported previously (1). The post-mitochondrial supernatant was layered over a step gradient of 22% and 37% (w/v) sucrose and centrifuged for 2 hrs at 136,000 x g (R_{max}). This procedure yielded a band of microsomes at the 22-37% interface free of mitochondrial membrane fragments as judged by the low activity of the mitochondrial marker enzyme cytochrome c oxidase.

ATPase activity was measured as the release of P_i. The reaction

media contained 50 mM Tris-HCl (pH 7.5), 2 mM ATP(Na_2), 2 mM $MgCl_2$, 10 mM KCl where indicated, 22 mM sucrose and 10 to 20 μg membrane protein in a final volume of 1 ml. The reaction was started by the addition of ATP, carried out at 37° C for 15 min. and stopped by adding 1 ml of 14% TCA. P_i was determined on the whole sample by the extraction of phosphomolybdate with butyl acetate and measuring the unreduced phosphomolybdate at 310 mμ according to the method of H. Sanui (6). The term "basal ATPase" refers to the activity observed when the only activating ion added was Mg^{++}.

Valinomycin, gramicidin D and CCCP (carbonyl cyanide, m-chlorophenyl hydrazone) were obtained commercially. Nigericin and trinactin were gifts from Dr. J. Berger of Hoffmann-LaRoche Inc. and Dr. V. Gooch of our Department, respectively.

RESULTS AND DISCUSSION In the course of our work with the K^+-stimulated ATPase of oxyntic cell microsomes, we observed that the K^+-dependent portion of the activity increased as the preparation was aged at 4° C. This must have contributed to the variability of the K^+-ATPase activity reported previously (1), i.e., from 7.5 to 20 μmoles P_i/mg·hr. It seemed possible that the enhancement of K^+-dependent activity with age might be the result of degenerative processes which break down membrane structure and increase the passive permeability to K^+, thereby allowing greater access of K^+ to some activating site. Indeed our initial attempts to alter K^+ permeability in fresh membranes revealed that the K^+ ionophoretic substances (7), valinomycin, nigericin, gramicidin, and trinactin, all markedly stimulated the K^+-dependent rate (Table I). The degree of stimulation has been as much as 5 fold although it varied somewhat with each preparation and with the particular ionophore employed. In the case of gramicidin, which was studied in greatest detail, the concentration for half-maximal stimulation was 1.6×10^{-7} M and for maximal stimulation, 1×10^{-5} M; in terms of the weight ratios of gramicidin to protein these values correspond to 0.016 and 1.0, respectively.

TABLE I

EFFECT OF VARIOUS IONOPHORES ON THE K^+-ATPase

Additions	ATPase Activity (μmoles/mg·hr)	
	Total	K^+-Dependent**
Basal Mg^{++}-ATPase	28.3	0
KCl (10 mM)	36.5	8.2
KCl + Nigericin (3×10^{-6}M)*	62.8	34.5
KCl + Trinactin(7×10^{-6}M)	57.8	29.5
KCl + Valinomycin (10^{-6}M)	45.2	16.9
KCl + Gramicidin (10^{-6}M)	52.3	24.0
KCl + CCCP (10^{-5}M)	39.3	11.0
KCl + CCCP (10^{-5}M) + Gramicidin (10^{-6}M)	53.7	25.4

* The ionophores were added in 1 to 10 μl of ethanol or acetone; none of these substances stimulated the basal ATPase activity.
** Activity in the presence of 10 mM KCl minus the basal rate.

TABLE II

EFFECT OF LYOPHILIZATION ON THE K^+-ATPase

Additions	Control	Lyophilized*
	(μmoles/mg·hr)	
Basal Mg^{++}-ATPase	15.1	15.2
KCl (10 mM)	22.5	47.7
KCl + Nigericin (10^{-6}M)	54.0	57.5
KCl + Gramicidin (10^{-6}M)	55.9	59.4

* Membranes were lyophilized in 0.3 M sucrose and 5 mM PIPES (Na) buffer, pH 6.8.

Since this membrane fraction may play some role in the H^+ transport function of the oxyntic cell, it was of interest that the H^+ ionophore, CCCP, had very little effect on the K^+-ATPase activity under these conditions (Table I). Furthermore, virtually no effect was observed on the enhancement produced by the more K^+-specific ionophores.

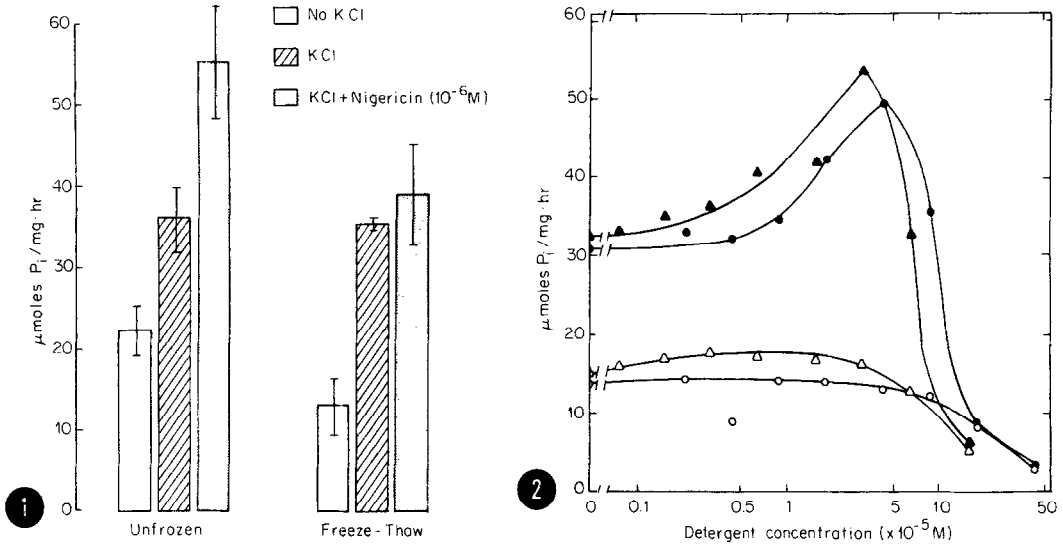

Fig. 1. Effect of repeated freezing and thawing on the K^+-stimulated ATPase and its ionophoretic activation. Values for the basal and K^+-stimulated rates represent the averages from 8 experiments; those with nigericin are the average of 3 experiments. Bars represent the S. E. M. Membranes in the ATPase reaction media (minus ATP) underwent 3 cycles of freezing in a dry ice-acetone bath and thawing in cold water.

Fig. 2. Effect of Lubrol PX and Triton X-100 on the basal and K^+-stimulated ATPase activities. Circles represent activity in the presence of Lubrol PX, with (●) and without (○) 10 mM KCl; the triangles indicate activity with Triton X-100, with (▲) and without (△) 10 mM KCl. The values are corrected for any detergent interference in the P_i assay; this was less than 10% at the point of complete inhibition of the K^+-dependent ATPase.

It is significant that the ATPase was activated only by K^+. Na^+ could not substitute nor could it stimulate the ATPase under any circumstance tested, either alone or in combination with K^+ or any ionophore. In fact, at higher concentrations of Na^+, 25-60 mM, the K^+-ATPase was inhibited slightly. Thus the presently studied K^+-ATPase is clearly distinguished from the widely studied (Na^+-K^+)-ATPase and the cation activated mitochondrial ATPase (8, 9).

If the ionophores enhance the K^+-ATPase by eliminating permeability barriers to K^+, then other treatments aimed at disrupting membrane vesicles should also be effective stimulants. The effects of lyophilization, repeated freezing and thawing, and detergents were studied in this regard. Lyophilization (Table II) had a pronounced stimulatory effect on the K^+

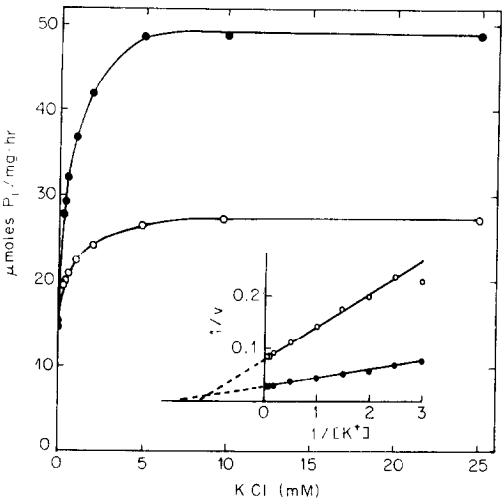

Fig. 3. Effect of gramicidin on the K^+ activation of the microsomal ATPase activity. Points represent activity with (●) and without (○) 10^{-6}M gramicidin.

dependent activity increasing it from 7.4 to 32.5 µmoles P_i/mg·hr, thus making it nearly equivalent to the ionophore expressed rate. Similar results were obtained after freezing and thawing (Fig. 1): the K^+-dependent portion of the ATPase activity increased 66% while nigericin had very little stimulatory effect after the freeze-thaw treatment.

Treatment of the membranes with the nonionic detergents Lubrol PX and Triton X-100 had a biphasic effect on the K^+-ATPase (Fig. 2). At low concentrations of detergent (from about 1 to 5 x 10^{-5}M) there was enhancement of the ATPase activity while at higher concentrations (greater than 10^{-4}M or detergent to protein ratios of 3:1) there was complete inhibition. It should be noted that a number of the nonionic detergents at sublytic concentrations appear to function as K^+-selective ionophores (10, 11). Thus the stimulation observed may be due to the ionophoretic nature of these substances rather than to dissolution of membrane constituents.

The results presented so far indicate that K^+ permeability is a limiting factor in the microsomal ATPase activity. It is apparent from the K^+ activation curves in Fig. 3 that this permeability limitation is not overcome by simply increasing K^+ concentration since, a) the activation followed

Michaelis-Menten saturation kinetics and not passive permeation kinetics, and b) the addition of ionophore was required for full activation. Thus V_{max} was increased as much as three fold by gramicidin, while there was but a slight shift in the estimated K_a for K^+ activation from 0.78 to 0.58 mM K^+ (Fig. 3). Such kinetics are consistent with a recruitment of new enzyme sites by the ionophore. Extending this to the permeability limitation existing in the gastric microsomal membranes, it is reasonable to interpret the present experiments in terms of two populations of membranes: one which is of sealed, K^+-impermeable vesicles and another freely permeable to K^+. The sealed membrane vesicles would be the source of the ionophore stimulated, K^+-dependent ATPase activity and the permeable membranes the source of the K^+-dependent rate measured in the absence of ionophores.

Present data do not allow us to distinguish between a model whereby the ATPase is simply activated by K^+, or one where activation is accompanied by cation translocation. Furthermore, such ion transport activity could either be an export from an enclosed vesicular space, thus depleting K^+ from the activating site, or it could be transport accumulation of K^+ resulting in a gradient-limited reduction in ATPase activity. Such distinctions will be possible with alternative modes of experimentation (e.g., ion specific electrodes) which should also help to determine the role of the K^+-stimulated ATPase in gastric oxyntic cell function.

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