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SPECIFIC MODIFICATION OF GASTRIC K^+-STIMULATED ATPase ACTIVITY BY THIMEROSAL

JOHN G. FORTE, JEAN L. POULTER, ROSS DYKSTRA, JOSE RIVAS and HON CHEUNG LEE

Department of Physiology-Anatomy, University of California, Berkeley, CA 94720 (U.S.A.)

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Treatment of hog gastric microsomes with the sulfhydryl reagent, thimerosal (ethylmercurithiosalicylate), produced differential effects on the K^+-ATPase and the K^+-stimulated *p*-nitrophenylphosphatase activities. For example, exposure to 2 mM thimerosal for 3 min severely reduced the activity of K^+-stimulated ATPase, while K^+-*p*-nitrophenylphosphatase activity was enhanced 2- to 3-fold. Higher concentration of thimerosal, or longer incubation times, also led to inhibition of K^+-*p*-nitrophenylphosphatase. The activated state of *p*-nitrophenylphosphatase could be sustained by a 20-fold, or greater, dilution of treated membranes, and could be reversed by reduction of membrane SH groups by exogenous thiols. Significant activation of K^+-*p*-nitrophenylphosphatase was not produced by *p*-chloromercuribenzene sulfonate, *p*-chloromercuribenzoate or mersalyl; however, ethyl mercuric chloride had qualitatively similar activity effects as thimerosal. Kinetics of K^+-*p*-nitrophenylphosphatase for thimerosal-treated membranes were altered as follows: V increased; K_m for *p*-nitrophenylphosphate unchanged for K_a for K^+ increased. ATP, which is a potent inhibitor of K^+-*p*-nitrophenylphosphatase activity in native membranes ($K_I \approx 200$ μM). These data suggest that there are multiple SH groups which differentially influence the gastric K^+-stimulated ATPase activity. Defined treatments with thimerosal are interpreted as an uncoupling of the K^+-stimulated phosphatase component of the enzyme (for which *p*-nitrophenylphosphatase is a presumed model reaction). Such differential modifications can be usefully applied to the study of partial reactions of the enzyme and their specific role in the related H^+-transport reaction.

Introduction

In previous reports we have described the features and properties of gastric microsomal ATPase activity that is dependent on Mg^{2+} and stimulated by K^+ with further stimulation by K^+-ionophores [1–3]. This so-called gastric K^+-stimulated ATPase is responsible for a H^+/K^+ exchange pump in the isolated microsomal vesicles [4–6], and may be the enzymic site for gastric H^+ transport [7,8]. The partial reactions for the gastric K^+-stimulated ATPase activity have also been studied in some detail and a plausible reaction sequence has been given [9,10]. The general scheme includes a Mg^{2+}-dependent kinase which catalyzes the phosphorylation of a 100-kdalton membrane protein. Phosphoprotein phosphatase activity is stimulated by K^+ and this partial reaction has often been studied using K^+-stimulated *p*-nitrophenylphosphatase activity (K^+-*p*-nitrophenylphosphatase) as a model system [2,3,11].

Compounds of mercury are known to react with sulfhydryl groups and lead to inactivation, and in some cases activation, of enzymatic systems [12–14]. Like many other ATPases, the gastric K^+-stimulated ATPase is sensitive to inhibition by compounds that react with sulfhydryl groups [1,6]. One interesting series of studies has focused on the inhibition of an analogous transport enzyme, $(Na^+ + K^+)$-ATPase, by ethylmercurithiosalicylate (thimerosal) [15–18]. Differential effects by thimerosal on the

Abbreviation: Pipes, piperazine-*N*,*N*'-bis(ethanesulfonic acid).

partial reactions of the $(Na^+ + K^+)$-ATPase have been interpreted as further evidence for the existence of two ATP binding sites within the functional enzyme [15].

In this study we have explored the effects of thimerosal on the partial reactions of the gastric K^+-stimulated ATPase. In particular we have shown that while total activity of K^+-stimulated ATPase was inhibited by thimerosal the K^+-*p*-nitrophenylphosphatase activity was stimulated 2- to 3-fold by certain treatment and more extensive treatment finally led to inhibition by the mercurial. Kinetic analysis of thimerosal treated membranes suggests that the K^+-stimulated activity had been altered at some critical intermediate step. The altered enzyme allows a much higher turnover of the model substrate, *p*-nitrophenyl phosphate, at the phosphatase site.

Materials and Methods

The membrane fractions used for this study were isolated from hog gastric mucosa by the procedure described previously [6]. Briefly, scrapings from the fundic mucosa of hog stomach were triturated in homogenization medium consisting of 113 mM mannitol, 37 mM sucrose, 0.2 mM EDTA and 5 mM Pipes buffer (pH 6.68). Microsomes were harvested by differential centrifugation and further purified by density gradient centrifugation. The light membrane fraction that banded between 20% and 27% sucrose was collected and immediately frozen for subsequent use in these studies.

For most of the experiments reported here the treatment with thimerosal consisted of incubating membranes (approx. 0.6–1.2 mg protein/ml) at 37°C in a solution containing 50 mM Tris (pH 7.4) and various concentrations of thimerosal; specific conditions are described for individual experiments. After a designated time of treatment the membranes were diluted 20- to 30-fold with homogenization medium and maintained at room temperature (21–23°C) until distributed into the various enzyme assay tubes. The properties of the modified enzyme were always compared with those of the control enzyme that had been carried through the same procedure in the absence of thimerosal.

Assay of *p*-nitrophenylphosphatase activity was carried out in a total volume of 1 ml containing 10 mM Tris buffer (pH 7.5), 5 mM $MgSO_4$, 5 mM *p*-nitrophenyl phosphate, 10 to 25 μg of membrane protein and always included parallel sets of tubes with and without 10 mM KCl. The enzyme incubation continued for 10 to 20 min at room temperature until the reaction was stopped by adding 1.5 ml 0.5 M NaOH. After a brief centrifugation to remove the consequent precipitate of $Mg(OH)_2$ the absorbance was measured at 410 nm. Enzyme activity in the absence of KCl was small (10% or less) compared to that measured in the presence of KCl; the difference in activity with and without KCl was used to express K^+-*p*-nitrophenylphosphatase activity.

In a few experiments *p*-nitrophenylphosphatase activity was monitored directly by recording absorbance at 410 nm as a function of time. Thimerosal, or other reagents, were added at designated times and the relative changes in rate were noted.

ATPase activity was measured as previously described [6]. The basal assay medium consisted of 1 mM $MgSO_4$, 1 mM ATP, 10–25 μg membrane protein, and 10 mM Pipes buffer in a reaction volume of 1 ml at pH 6.7. Enzyme activity in the basal medium was compared with that measured in the presence of 140 mM KCl and in the presence of 140 mM KCl plus 10^{-5} M valinomycin. After proceeding for 10 to 20 min at room temperature the enzyme reaction was stopped by the addition of 1 ml of cold 15% CCl_3COOH. Assay of inorganic phosphate was carried out by the method of Sanui [19].

Results

A dose response curve for the effects of thimerosal on gastric microsomal ATPase activity is shown in Fig. 1A. The results of a typical experiment assaying ATPase activities after varying time of exposure to a fixed dose of 2 mM thimerosal are shown in Fig. 1B. K^+-dependent ATPase activity, especially the component of K^+-ATPase that was further enhanced by valinomycin, was more sensitive to inhibition by thimerosal than the ATPase activity in the absence of K^+. This pattern of enzyme sensitivity is generally similar to what has been reported for other SH reagents [6].

Contrary to what was observed for ATPase activity the effects of thimerosal on K^+-*p*-nitrophenylphosphatase showed characteristics of activation of enzyme activity as well as inhibition. The dose

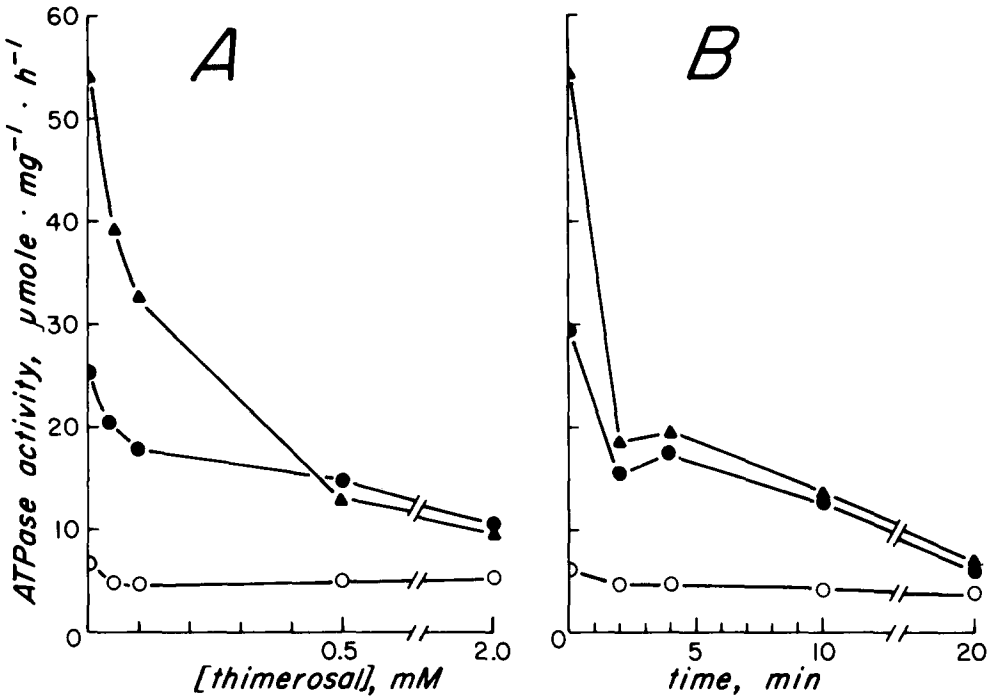

Fig. 1. Effect of thimerosal on gastric microsomal ATPase activities. Gastric microsomes were first pre-incubated with thimerosal as described in the Methods at the indicated concentration and time. The reaction was quenched by a 1 : 20 dilution with H_2O and samples were apportioned for measurement of ATPase activity with Mg^{2+} only (○), with Mg^{2+} plus 140 mM KCl (●), or with Mg^{2+} plus 140 mM KCl plus 10^{-5} M valinomycin (▲). A. Pre-incubation conditions were all carried on for 10 min at 37°C at the concentration of thimerosal indicated. B. Preincubation was carried out at 37°C using 2 mM thimerosal for all samples with the time being as shown.

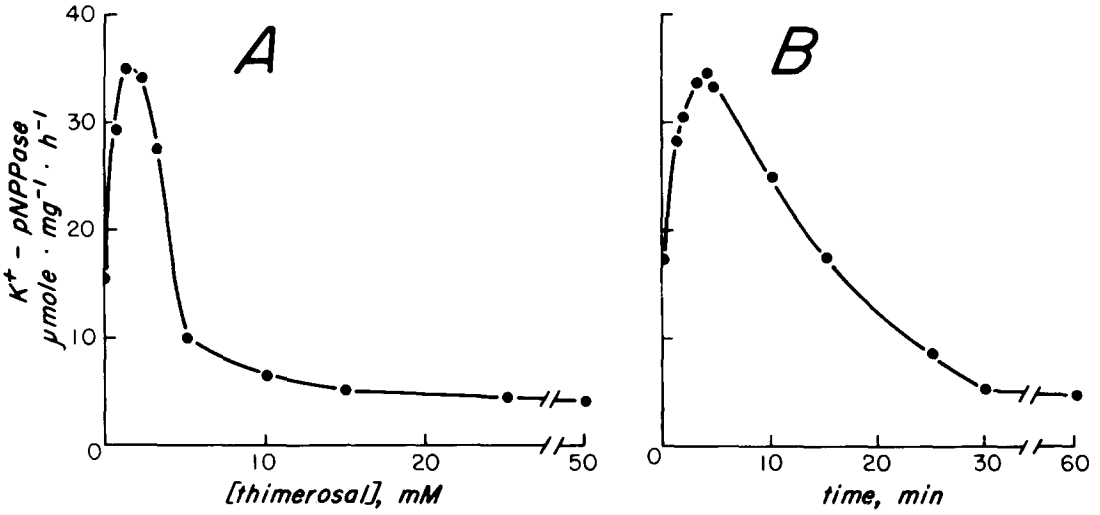

Fig. 2. Effect of thimerosal on gastric microsomal K^+-stimulated *p*-nitrophenylphosphatase (pNPPase) activity. Gastric microsomes were first preincubated with thimerosal as described in the Methods and at the indicated concentration and time. The reaction with thimerosal was quenched by 1 : 20 dilution with H_2O and the K^+-*p*-nitrophenylphosphatase was measured in aliquots of the treated membranes. A. Pre-incubation with the indicated concentration of thimerosal were all carried out for 3 min at 37°C. B. Pre-incubation was carried out using 1.5 mM thimerosal at 37°C and at the various times as indicated.

response curve for thimerosal is shown in Fig. 2A; the effects of time exposure to 2 mM thimerosal are given in Fig. 2B. With relatively gentle treatment (e.g., 2 mM thimerosal for 3 to 5 min) there was an increase in K^+-stimulated phosphatase activity in the range of 100–200%, while greater time of exposure and/or concentration of the mercurial led to reduced enzyme activity.

In testing the effect of pH on the activation produced by thimerosal we found a rather broad range of pH at which the enzyme could be incubated with thimerosal to produce the activation of the K^+-*p*-nitrophenylphosphatase (pH 6.5–10). For convenience, pH 7.5 was chosen as the standard condition for thimerosal treatment.

Since there were progressive alterations in activity as the membranes were incubated with thimerosal, it was necessary to establish conditions under which the progression of change could be stopped. The effects of quenching by dilution are shown in Fig. 3. The activation of K^+-*p*-nitrophenylphosphatase produced by thimerosal was stable for up to 48 h if the membranes were diluted immediately after treatment and stored at −20°C. When the treated membranes were diluted and stored at room temperature (22–23°C), the activated K^+-*p*-nitrophenylphosphatase was relatively stable for 1 to 2 h, whereas rapid inactivation of enzyme activity occurred when membranes were maintained at 37°C after dilution. These results suggest that there is continued reaction with thimerosal and/or enzymatic decay after a 20-fold dilution. However, the relatively slow rate of change observed for preparations diluted at room temperature permitted the handling required to set up and run our typical assay conditions (approx. 20 to 30 min).

Several other organic mercurials were tested in a search for comparative effects on the activation of K^+-*p*-nitrophenylphosphatase. The results for *p*-chloromercuribenzene sulfonate, *p*-chloromercuribenzoate and mersalyl suggest little or no significant activation and the dose response for inhibition was similar for these three reagents (Fig. 4). Dithiobis-(nitrobenzoic acid) also produced inhibition without a significant activation of K^+-*p*-nitrophenylphospha-

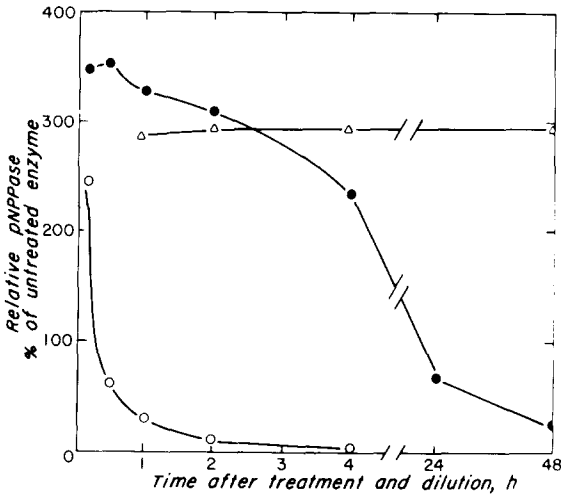

Fig. 3. Effect of dilution on the relative K^+-*p*-nitrophenylphosphatase (pNPPase) activity of thimerosal-treated membranes. Membranes were pre-treated with 2.5 mM thimerosal for 3 min at 37°C then diluted with H_2O and maintained at various temperatures. Dilution temperatures were as follows: immediate freezing at −20°C (△), room temperature (●), and 37°C (○). Samples were taken from the diluted membranes at the times indicated and K^+-*p*-nitrophenylphosphatase activity was assayed. Control preparations not treated with thimerosal, were run in parallel and the enzyme activity was measured and assigned the value of 100% activity.

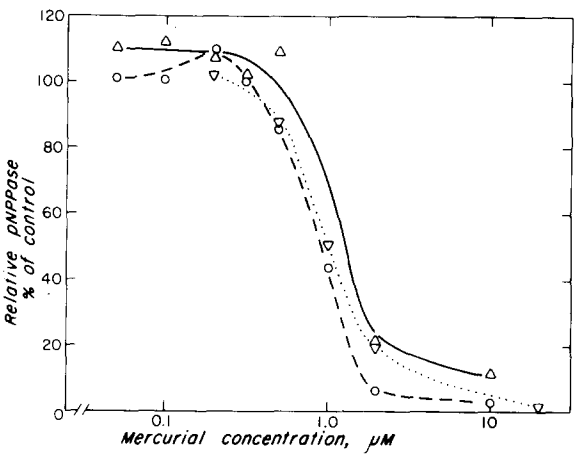

Fig. 4. Effect of various mercurials on relative K^+-*p*-nitrophenylphosphatase (pNPPase) activity of gastric microsomes. Membranes (29 μg/ml) were incubated in a cuvette as described for assay conditions in the Methods. The reaction was followed spectrophotometrically at 410 nm to establish the control rate (100%). Mercurial reagents were then added at the indicated concentration and the change in reaction rate monitored; for above reagents the changes were complete (i.e., steady rate) by 10 min after addition. △, *p*-chloromeruribenzoate; ○, *p*-chloromercuribenzenesulfonate; ▽, mersalyl.

TABLE I

REVERSAL OF THIMEROSAL EFFECTS BY THIOLS

Microsomes (1.2 mg protein/ml) were first treated at 37°C for 3 min with the indicated reagents. Thiols or water were then added as indicated and the incubation continued for 1 min. The preparations were then diluted with water (1 : 20 in one experiment and 1 : 10 in another experiment) and apportioned for assay of K^+-*p*-nitrophenylphosphatase. Values are reported as the % of the control, untreated, enzyme (±S.E.) for the particular experiment.

Treatment		K^+-*p*-nitrophenylphosphatase	
1st	2nd	% of control	*n*
Control	H_2O	100	4
1 mM thimerosal	H_2O	331 ± 0.5	2
1 mM thimerosal	2 mM thio-salicylate	117 ± 25	2
1 mM thimerosal	2 mM β-mercaptoethanol	113 ± 2	2
1 mM thiosalicylate	H_2O	108 ± 16	4
2 mM thiosalicylate	H_2O	113 ± 7	4

tase (data not shown). On the other hand, methyl mercuric chloride produced a pattern of stimulation and inhibition similar to thimerosal, but the required doses were about 100-fold less (Fig. 5). The action of thimerosal in stimulating (or inhibiting) K^+-*p*-nitrophenylphosphatase activity was reversed by thiols such as β-mercaptoethanol, dithiothreitol or even by an excess of thiosalicylate (Table I). Thiosalicylate (2 mM) had no effect of its own in altering the observed K^+-*p*-nitrophenylphosphatase activity. Membranes first treated with *N*-ethylmaleimide became

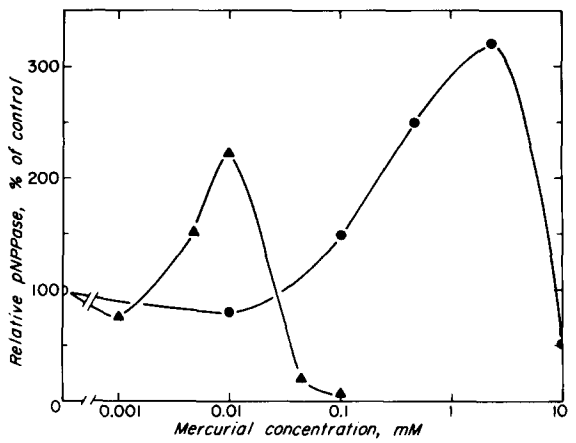

Fig. 5. Effect of thimerosal and methyl mercuric chloride on activation and inhibition of gastric microsomal K^+-*p*-nitrophenylphosphatase (pNPPase) activity. Conditions of assay were as described in Fig. 4. Reaction rates were monitored 10 min after adding the indicated concentration of reagent and compared to the rate 100% prior to addition. ▲, methyl mercuric chloride; ●, thimerosal.

TABLE II

EFFECTS OF *N*-ETHYLMALEIMIDE AND THIMEROSAL ON K^+-*p*-NITROPHENYLPHOSPHATASE (K^+-pNPPase) AND ATPase ACTIVITIES

Microsomes (2.2 mg protein/ml) were first treated for 5 min at 37°C with the indicated concentration of *N*-ethylmaleimide (NEM) or with no added agent. Then 2 mM thimerosal was added to the tubes indicated and incubation proceeded at 37°C for an additional 4 min. All preparations (control and treated enzyme) were then diluted 1 : 20 with distilled water at room temperature and aliquots were apportioned for enzyme assays. Enzyme activities are reported as the K^+-stimulated rates, i.e., activity stimulated by K^+ over that without K^+, ±S.E. of four measurements from two separate experiments. Conditions for assay were as given in the Methods; tubes for ATPase assay included 10^{-5} M valinomycin.

	Activity ($\mu mol \cdot mg^{-1} \cdot h^{-1}$)	
	K^+-pNPPase	K^+-ATPase
Control	10.2 ± 0.9	12.2 ± 1.5
H_2O, then thimerosal	29.8 ± 0.5	5.6 ± 1.0
1 mM NEM	10.6 ± 0.4	6.8 ± 1.2
1 mM NEM, then thimerosal	9.8 ± 1.0	0.5 ± 0
5 mM NEM	4.5 ± 0.5	1.5 ± 0.2
5 mM NEM, then thimerosal	4.1 ± 0.6	0.1 ± 0.03

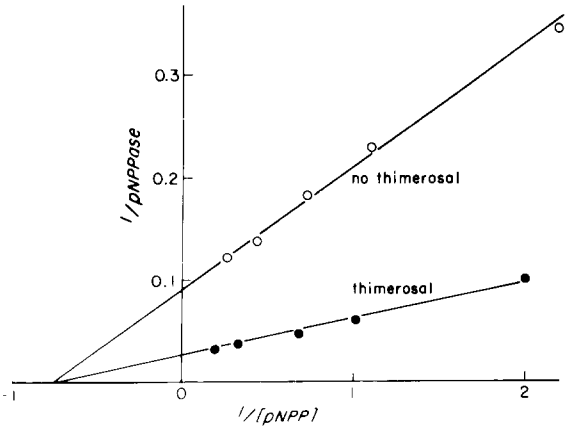

Fig. 6. Kinetics of *p*-nitrophenylphosphatase (pNPPase) reaction in untreated and thimerosal-treated membranes. Membranes were pre-incubated with or without 2 mM thimerosal for 3 min at 37°C. After dilution, the membranes were apportioned to tubes containing different concentrations of *p*-nitrophenyl phosphate and the enzyme activity was measured. The results are plotted as a double reciprocal plot where *p*-nitrophenyl phosphate is given in mM and *p*-nitrophenylphosphatase activity is in μmol · mg^{-1} · h^{-1}.

TABLE III

EFFECT OF K^+ ON KINETIC PARAMETERS OF GASTRIC MICROSOMAL K^+-*p*-NITROPHENYLPHOSPHATASE ACTIVITY

Values for K_m, V and K_a are shown for the mean ±S.E. with the number of experiments indicated in parentheses. The K_i values were determined from an individual experiment in which the concentrations of the respective inhibitor and substrate (*p*-nitrophenyl phosphate) was varied as exemplified for ATP in Fig. 8.

	Native enzyme	Thimerosal-treated
K_m (*p*-nitrophenyl-phosphate, mM)	1.44 ± 0.14 (5)	1.57 ± 0.13 (4)
V (μmol · mg^{-1} · h^{-1})	13.6 ± 0.5 (5)	42.5 ± 1.4 (4)
K_a (K^+, mM)	4.3 ± 0.2 (2)	0.9 ± 0.1 (2)
K_i, (ATP, mM)	0.012 (mixed)	0.2 (comp)
K_i, (ADP, mM)	0.12 (comp)	0.28 (comp)
K_i, (P_i, mM)	1.0 (comp)	0.83 (comp)

refractory to the stimulation of K^+-*p*-nitrophenylphosphatase typically produced by thimerosal, yet the mercurial reatained its effectiveness on the inhibition of K^+-ATPase (Table II).

The effects of thimerosal in altering the kinetics of enzymic interaction with substrate are shown in Fig. 6 and summarized in Table III. The K_m for *p*-nitrophenyl phosphate was not significantly altered by the treatment with this mercurial ($P < 0.05$), whereas under the specified condition V had increased by about 3-fold. The kinetics for activation of *p*-nitrophenyl phosphatase activity by K^+ were distinctly altered by treatment with thimerosal (Fig. 7). The measured K_a for K^+ was decreased from 4.3 ± 0.2 mM in control membranes to 0.9 ± 0.1 mM in thimerosal-treated preparations (Table III).

ATP and other phosphates have been shown to be effective inhibitors of gastric K^+-*p*-nitrophenylphosphatase activity [11]. Kinetics for inhibition of the control enzyme activity by ATP are shown in Fig. 8A. From the analysis of similar double reciprocal plots for three experiments, we conclude that inhibition of K^+-*p*-nitrophenylphosphatase activity is of a mixed type. The calculated K_I for ATP on the native enzyme was 12 μM. After activation of K^+-*p*-nitrophenylphosphatase activity by thimerosal the kinetics of inhibition by ATP changed markedly (Fig. 8B); in this case, ATP now appeared to be a purely competi-

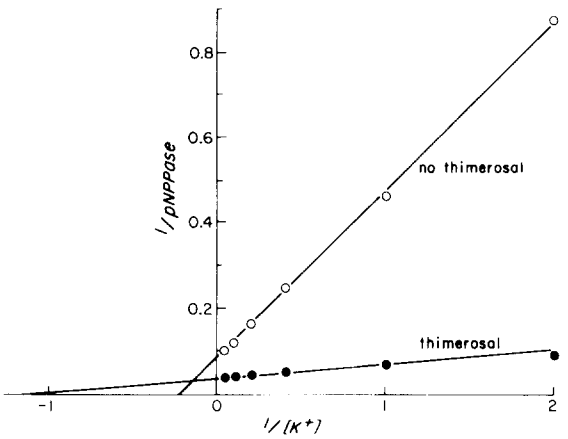

Fig. 7. Change in kinetics of activation by K^+ for control and thimerosal-treated membranes. Pre-incubation conditions were as described in Fig. 6. All assay tubes contained 5 mM *p*-nitrophenyl phosphate but with varying concentrations of K^+. Results are plotted as a double reciprocal plot with [K^+] being given in mM and *p*-nitrophenylphosphatase (pNPPase) in μmol · mg^{-1} · h^{-1}.

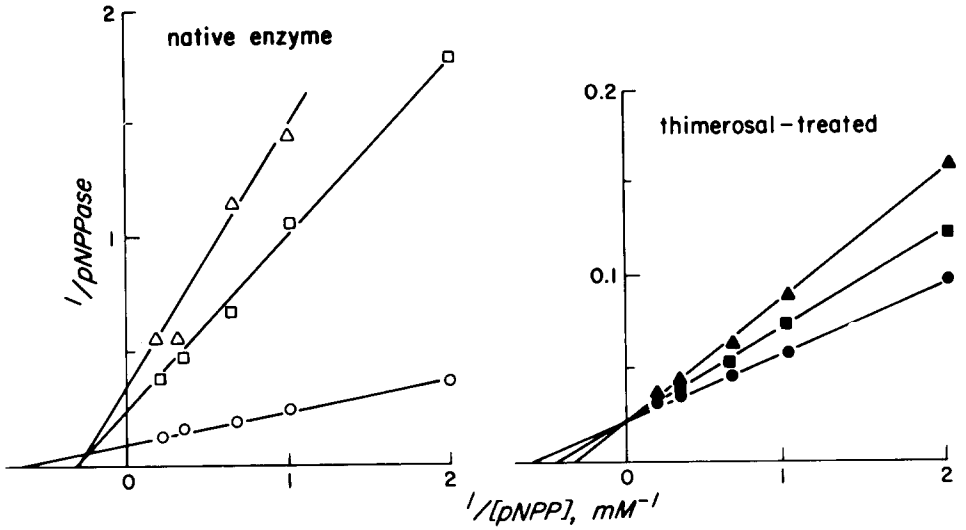

Fig. 8. Kinetics of inhibition by ATP on *p*-nitrophenylphosphatase (pNPPase) activity of control and thimerosal-treated gastric microsomal membranes. Pre-incubation conditions were as described in Fig. 6. After treatment, diluted membranes were added to assay tubes containing various concentrations of *p*-nitrophenyl phosphate (pNPP), and with or without ATP. No ATP (○, ●); 0.05 mM ATP (□, ■); 0.1 mM ATP (△); 0.2 mM ATP (▲). The results are plotted as a double reciprocal plot.

tive inhibitor and sensitivity was much reduced, with a K_I of 200 μM. The effects of ADP and inorganic phosphate on K^+-*p*-nitrophenylphosphatase activity were tested on native and thimerosal-treated enzyme (Table III). Both agents appear to act as simple competitive inhibitors for the native enzyme as well as for the thimerosal-treated enzyme.

Discussion

Kinetic and inhibition studies have been employed toward understanding the reaction mechanism of the gastric K^+-stimulated ATPase and its possible role in HCl secretion [6,10,20–22]. These studies have provided a plausible sequence for the reaction mechanism as follows:

$$A + ATP \xrightarrow{Mg^{2+}} E{-}ATP \quad (1)$$

$$E{-}ATP \xrightarrow{Mg^{2+}} E{-}P + ADP \quad (2)$$

$$E{-}P \xrightarrow{K^+} E + P_i \quad (3)$$

The first step is the binding of ATP to the reaction site. The second step involves a Mg^{2+}-dependent kinase activity with the formation of a phosphoprotein intermediate, E-P [9,10,22]. Finally, the intermediate is hydrolyzed via a K^+-dependent phosphoprotein phosphatase.

The reaction of the organic mercurial, thimerosal, with the gastric K^+-ATPase system is complex, involving distinctive stages of inhibition and activation. A most striking response was the activation of K^+-*p*-nitrophenylphosphatase activity even when most of the K^+-stimulated ATPase activity (and H^+ transport) was inhibited. Further reaction with thimerosal finally led to inhibition of K^+-*p*-nitrophenylphosphatase activity. This complexity of enzymatic response suggests that several types of sulfhydryl groups can be independently modified producing total inhibition, selective inhibition or an apparent uncoupling of the total reaction sequence given above. These changes are analogous to what has been observed in the related transport enzyme, $(Na^+ + K^+)$-ATPase [15–18].

Although K^+-ATPase activity is clearly reduced under conditions where phosphatase activity is markedly elevated, we feel that the present data are still best interpreted in terms of *p*-nitrophenyl phosphate being hydrolyzed via the reaction scheme shown above and not by a second, independent, enzyme. We propose that there is a class of thimerosal-sensitive SH groups whose reaction with ethyl mercury makes

the phosphatase site very highly reactive with *p*-nitrophenyl phosphate, e.g., by increasing the number or turnover of effective sites. At the same time, the intermolecular transfer of energy associated with phosphate bond transfer and the interdependent H^+-transport step are blocked. When these latter reactions occur in the native enzyme they are much preferred, thus ATP, or more likely the resulting phosphoenzyme intermediate, is ordinarily an effective inhibitor of K^+-*p*-nitrophenylphosphatase activity. After the enzyme has been treated with thimerosal, ATP is a relatively poor inhibitor, simply competing as a phosphate directly at the *p*-nitrophenyl-phosphate site. Essentially, this proposal predicts an uncoupling of the complex system of ATP utilization and hydrolysis from the more simple phosphate ester cleavage. The latter system retains its requirement for K^+; in fact, the affinity for K^+ is significantly increased in the thimerosal-treated preparations.

Differential effects of modifying reagents on physiological activity are usually interpreted in terms of variations in accessibility of the drug to the particular functional group. Criteria for selectivity include size, charge, partition coefficient and reactivity. Thimerosal may be a particularly gentle, or selective, reagent because of its own thiol buffering power. That is, the ability of the protein SH group to compete with the endogenous thiosalicylate. By virtue of their distinctive characteristics of dissociating K^+-*p*-nitrophenylphosphatase from gastric K^+-ATPase activity, thimerosal and methyl mercuric chloride can be differentiated from the other SH reagents tested. This result is consistent with the observations of Henderson et al. [17] for the $(Na^+ + K^+)$-ATPase. Thimerosal, methyl mercury and ethyl mercury (see Ref. 17) are selectively accessible to some site in a reaction sequence, such as that shown as 1–3 above, that would still permit the phosphatase activity of the enzyme while blocking total flow (ATPase) through the system. The increased rate of phosphatase activity could be due to some minor structural change or relaxation within the enzyme. Other SH reagents may also react with this specific site, but if they have additional, and perhaps more prominent, effects on groups required for phosphatase activity then one would see parallel changes in phosphatase and ATPase activities. *N*-Ethylmaleimide may react with the same SH site as thimerosal since the alkylating reagent blocks the phosphatase activating effect of the mercurial. Moreover, *N*-ethylmaleimide shows very little activation effects of its own. This may be due to the nature of the product formed. That is, the site alteration is more restrictive than when occupied by ethyl or methyl mercury. Or there may be additional sites of reaction with *N*-ethylmaleimide that prevent the activation.

Earlier studies with dithiobis(nitrobenzoic acid) modification showed that a total of about 80 nmol of SH groups are present per mg of gastric microsomal membrane protein [6]. Of this total, about 50 nmol SH per mg protein were readily accessible to the reagent (i.e., did not require membrane solubilization) and their reaction appeared to be associated with inhibition of K^+-stimulated ATPase activity. Knowing that the dominant molecular weight of microsomal proteic components is about 100 k daltons [23], it is clear that there are a number of possible SH groups that may be differentially modified.

The general use of group modifying reagents has been of value in assessing a great number of enzymatic functions, and their application to the gastric K^+-stimulated ATPase has been no exception. Thus, it has been shown that an amino group [20] and a guanidino group [20,21] are near the ATP binding site. The present studies show that there are several classes of sulfhydryl groups that can be differentially modified. In the case of thimerosal, these alterations have usefully amplified the role of partial reactions in the complex operation of the K^+-stimulated ATPase. Experiments are presently underway to evaluate other partial reactions (e.g., ATP binding, ATP-ADP exchange, formation of phosphoenzyme intermediate) in an attempt to further characterize their operation and the influence of essential sulfhydryl groups in the K^+-stimulated ATPase and proton transport activities.

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