Biochimica et Biophysica Acta, 770 (1984) 65–72
Elsevier

BBA 71997

INHIBITION OF ($H^+ + K^+$)-ATPase AND H^+ ACCUMULATION IN HOG GASTRIC MEMBRANES BY TRIFLUOPERAZINE, VERAPAMIL AND 8-(*N*,*N*-DIETHYLAMINO)OCTYL-3,4,5-TRIMETHOXYBENZOATE

WHA BIN IM [a], DAVID P. BLAKEMAN [a], JOHN MENDLEIN [b] and GEORGE SACHS [b]

[a] *Department of Experimental Sciences, The Upjohn Company, Kalamazoo, MI 49001 and* [b] *Center for Ulcer Research and Education, VA Wadsworth Hospital Center, Los Angeles, CA 90073 (U.S.A.)*

(Received March 21st, 1983)
(Revised manuscript received September 20th, 1983)

Key words: H^+ accumulation; ($H^+ + K^+$)-ATPase inhibition; Trifluoperazine; Verapamil; Tertiary amine; (Hog stomach)

The mechanism of gastric antisecretory action for trifluoperazine, verapamil and 8-(*N*,*N*-diethylamino)octyl-3,4,5-trimethoxybenzoate (TMB-8) has been studied utilizing isolated hog gastric membranes enriched with ($H^+ + K^+$)-ATPase. The drugs inhibited the gastric ATPase due to their apparent competition with K^+ for the luminal high-affinity K^+-site of the ATPase. The dose to inhibit 50% (ID_{50}) of the ATPase in the membranes rendered freely permeable to K^+ (20 mM) was 50 μM for trifluoperazine and 1.5 mM for verapamil and TMB-8. In intact hog gastric membranes which develop a pH gradient in the presence of valinomycin, ATP and KCl, however, trifuoperazine at 4 μM, verapamil and TMB-8 at 15 μM inhibited 40 and 30% of the valinomycin-stimulated ATPase activity, respectively, and also blocked the ionophore-dependent intravesicular acidification as measured by aminopyrine accumulation. The enhanced potency of the drugs to inhibit the ATPase in the intact membrane vesicles may be attributed to the accumulation of the drugs as a weak base within the vesicles, where the luminal K^+-site of the ATPase is accessible. Calmodulin and Ca^{2+} had no effect on the extent of H^+-accumulation as measured by aminopyrine accumulation in the membrane vesicles which were prepared in the presence of 1 mM EGTA. Since the drugs showed similar potency in interfering with H^+ movements either in the membrane vesicles or isolated rabbit gastric glands stimulated by dibutylryl cAMP, it is reasonable to suggest the inhibitory effect of the drugs on ($H^+ + K^+$)-ATPase as a primary cause for such interferences in both cases. A trifluoperazine analog and other lipophilic amine drugs similarly inhibited ($H^+ + K^+$)-ATPase and H^+ accumulation in the membrane vesicles or in the glands. We have concluded that a tertiary amine, the only common functional group among these drugs, is primarily responsible for their ability to interact with the high-affinity K^+ site of the gastric ATPase.

Introduction

Trifluoperazine, an antipsychotic drug, is known as an inhibitor of calmodulin [1–3]. Verapamil and TMB-8, antiarrhythmic agents, have been reported to be Ca^{2+} antagonists [4–6]. The effect(s) of these drugs on gastric acid secretion is of considerable interest, since intracellular Ca^{2+} and calmodulin are known to affect secretory processes in endocrine and exocrine tissue [3–7]. Recently, trifluoperazine has been shown to inhibit gastric acid secretion in frog gastric mucosa as measured by a pH electrode and in isolated rabbit gastric glands

Abbreviations: TMB-8, 8-(*N*,*N*-diethylamino)octyl-3,4,5-trimethoxybenzoate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; Pipes, piperazine-*N*,*N*'-bis(2-ethanesulfonic acid).

stimulated with histamine or dibutyryl cAMP as measured by aminopyrine accumulation [8,9]. Also, we have observed potent antisecretory activity of verapamil and TMB-8 in the dibutyryl cAMP-stimulated glands. It is not clear, however, whether the antisecretory activity of these drugs is related to their antagonistic action on calmodulin or Ca^{2+}. Several recent studies [10,11] have pointed out possible interactions of phenothiazines and related compounds with cellular components other than calmodulin.

This investigation was initiated to gain some insight on the mechanism of gastric antisecretory action of trifluoperazine, verapamil and TMB-8. The studies with the glands indicated that the drugs act on the acid secretory step(s) distal to cAMP [9]. Since gastric $(H^+ + K^+)$-ATPase represents an enzyme involved in the terminal steps of the acid secretory process [12,13], we have examined the effect of the drugs on the gastric ATPase and on H^+ accumulation in isolated hog gastric membrane vesicles using aminopyrine as an internal pH probe. In this report, we will provide evidence that these drugs inhibit gastric $(H^+ + K^+)$-ATPase and H^+ accumulation in the membrane vesicles not due to their effect on calmodulin or Ca^{2+}, as generally suggested [8,9], but due to their interaction with the luminal K^+-site of the ATPase in competition with K^+. The common functional group in the drugs eliciting such competition appeared to be a tertiary amine, since many other lipophilic amines inhibited $(H^+ + K^+)$-ATPase and at the same time blocked aminopyrine accumulation in the glands.

Methods and Materials

Preparation of hog gastric membranes enriched in $(H^+ + K^+)$-ATPase. The mucosal scrapings from hog stomachs were suspended in about 10 vol. buffer 1 (250 mM sucrose/2 mM $MgCl_2$/1 mM EGTA/2 mM Hepes-Tris, pH 7.4), and were homogenized with 20 strokes of a motor-driven (1500 rpm) Teflon pestle in a Potter-Elvehjem homogenizer. The microsomal membranes were obtained from the mucosal homogenate by differential centrifugation [14] and suspended in sucrose-EGTA buffer. The membrane suspension was layered over a sucrose gradient consisting of 30 and 40% (w/w) sucrose and centrifuged for 1 h at $100\,000 \times g$. The membrane band above 30% sucrose was collected and stored under liquid nitrogen in the presence of 20% glycerol. The specific activity of $(H^+ + K^+)$-ATPase in the final fraction ranged from 90 to 110 μmol/h per mg protein.

To render the membrane vesicles freely permeable to cations, the gastric membranes were either lyophilized [15] or homogenized for 3 min with a Sorvall Omni-Mixer and freeze-thawed twice in the absence of glycerol.

Preparation of rabbit gastric glands. The glands were prepared by the method of Berglindh and Obrink [16] using a male New Zealand albino rabbit, weighing 1.5 to 2.5 kg. The digestion buffer contained 70 units collagenase (Type III, Millipore), 0.25 mg soybean trypsin inhibitor, 2 mg rabbit albumin and 2 mg D-glucose per ml of the buffer.

Assay procedures. $(H^+ + K^+)$-ATPase activity was determined in 1 ml incubation medium containing 40 mM Tris acetate, pH 7.4/180 mM sucrose/2 mM $MgCl_2$/2 mM ATP, with or without 20 mM KCl. Leaky gastric membranes were utilized unless stated otherwise. The reaction was for 8 min at 37°C and was terminated by adding 1 ml of 10% trichloroacetic acid and 0.1 g of HCl-washed charcoal. After removal of charcoal, inorganic phosphate released was measured by the method of Tsai et al. [17].

K^+-dependent *p*-nitrophenylphosphatase was assayed in 1 ml incubation medium containing 10 mM Tris-HCl, pH 7.5/5 mM $MgSO_4$/5 mM *p*-nitrophenyl phosphate, with or without 10 mM KCl. The reaction was terminated with 1.5 ml 0.5 M NaOH after incubation at 37°C for 5 min. The mixture was centrifuged briefly and the absorbance of the supernatant, at 410 nm, was measured. In all the assays performed under these conditions, reaction rates did not deviate from linearity with respect to reaction time and protein concentrations.

The distribution ratio of amino[^{14}C]pyrine was measured to monitor the degree of H^+ accumulation. The level of aminopyrine accumulated in the glands was determined as described by Berglindh [18]. In the isolated membrane vesicles, the aminopyrine uptake studies were carried out under

the following conditions. An aliquot, 10 μl, of membrane suspension in sucrose buffer (approx. 30 μg protein) with or without 5 μg valinomycin was mixed with 500 μl of a buffer containing 150 mM KCl/1 mM $MgSO_4$/10 mM Pipes, pH 7.0/1 mM ATP, and 3 μM amino[^{14}C]pyrine. The incubation was at 22°C for an indicated time. The rapid filtration using a Millipore filter (HAWP 0.45 μm) was employed to determine the amount of amino[^{14}C]pyrine trapped within the vesicles.

Phosphorylation of ($H^+ + K^+$)-ATPase was carried out with lyophilized hog gastric membranes in a medium containing about 50 μg protein/5 μM [γ-^{32}P]ATP/2 mM Mg^{2+} or Ca^{2+}, and 50 mM Tris-HCl, pH 7.4. The reaction was at 0°C. The termination of the reaction and the filtration of the reaction mixture over a Millipore filter (3 μm pore size) were carried out as described [15]. Protein was determined by the method of Lowry et al. [19] using bovine serum albumin as a standard.

Materials. The sources of the compounds studies were as follows: trifluoperazine: Sigma; verapamil: Knoll Pharmaceuticals; 8-*N*,*N*-diethylamino)octyl-3,4,5-trimethoxybenzoate (TMB-8): The Upjohn Co.; azacosterol (20,25-diazacholesterol): G.D. Searle; iprindole: Wyeth Laboratories; 1-[3-(dimethylamino)propyl]indol: Biological Screening Office (The Upjohn Co.); chlorpromazine and plasmocid: commercial sources. Amino-[^{14}C]pyrine and [γ-^{32}P]ATP were purchased from New England Nuclear. All other materials were of regent-grade quality and obtained from standard sources. Calmodulin was obtained from Caabco Inc. (Houston, TX).

Results

Trifluoperazine, verapamil and TMB-8 inhibited ($H^+ + K^+$)-ATPase activity in isolated hog gastric membranes. The dose to inhibit 50% (ID_{50}) of K^+-dependent ATP hydrolysis in the presence of 20 mM KCl was 50 μM for trifluoperazine and 1.5 mM for verapamil and TMB-8. Since the membranes used in this study were freely permeable to K^+, the ATPase was fully activated by 20 mM KCl without valinomycin or gramicidin. Fig. 1 shows Lineweaver-Burk plots of $1/[K^+]$ (0.2 to 2 mM) versus a reciprocal of the rate of K^+-dependent ATP hydrolysis in the absence or presence of

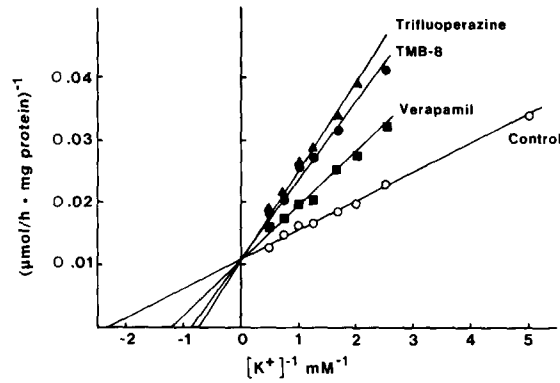

Fig. 1. Lineweaver-Burk plots of the rate of K^+-dependent ATP hydrolysis versus the concentration of K^+ varying from 0.2 to 2 mM in the absence (○) or presence of 15 μM trifluoperazine (▲), 0.4 mM verapamil (■) and 0.5 mM TMB-8 (●). Hog gastric membranes used in these experiments were made freely permeable to K^+. The activity of ($H^+ + K^+$)-ATPase was determined as the difference in ATP hydrolysis with or without K^+ at indicated concentrations. The V_{max} was 89 μmol/h per mg protein.

15 μM trifluoperazine, 0.4 mM verapamil or 0.5 mM TMB-8. The plots clearly showed that the K_m of K^+, 0.43 mM, was increased two to three times in the presence of the drugs while the V_{max}, 89 μmol/h per mg protein, was not affected. These data are consistent with competitive inhibition of the ATPase by the drugs with respect to K^+. Fig. 2

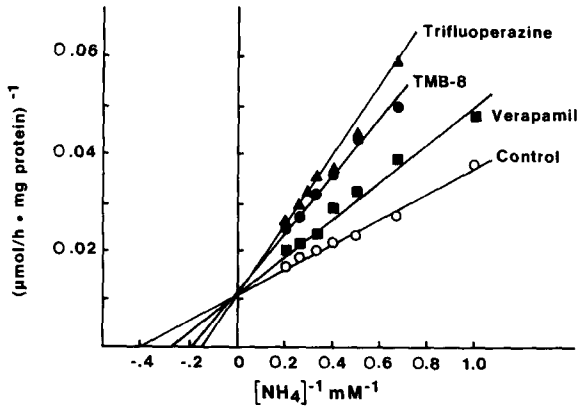

Fig. 2. Lineweaver-Burk plots of the rate of NH_4^+-dependent ATP-hydrolysis as a function of NH_4^+ concentration (1 to 5 mM) in the absence (○) or presence of 15 μM trifluoperazine (▲), 0.4 mM verapamil (■) or 0.5 mM TMB-8 (●). The V_{max} was about 86 μmol/h per mg protein. Other experimental conditions were the same as described in the legend of Fig. 1.

shows similar plots with the concentration of NH_4^+ (instead of K^+) varying from 0.5 to 5 mM. Again the principal effect of the drugs was to increase the K_m for NH_4^+; the value of K_m was 2.5 mM without the drugs, 6.4, 4.8 or 5.4 mM with 15 μM trifluoperazine, 0.4 mM verapamil or 0.5 mM TMB-8, respectively.

The effect of the drugs on phosphorylation of $(H^+ + K^+)$-ATPase in lyophilized hog gastric membranes was examined in the presence of 5 μM [γ-^{32}P]ATP and 2 mM $MgCl_2$ at 0°C. Trifluoperazine at 80 μM, verapamil or TMB-8 at 1 mM reduced the level of phosphorylated intermediate at equilibrium (50 s) to less than 10% of the control. Similar studies were carried out using 2 mM $CaCl_2$ instead of $MgCl_2$ since the Ca^{2+} form of phosphorylated enzyme is known to be more stable than the Mg^{2+} form of phosphorylated enzyme [20]. Again the drugs diminished the amount of phosphorylated enzyme at equilibrium (Fig. 3); almost 70% reduction by trifluoperazine (80 μM) and 80 to 90% reduction by verapamil and TMB-8 (1 mM). Fig. 4 shows that trifluoperazine at 80 μM, when added at equilibrium (6 min after the initiation of phosphorylation), had no noticeable effect on the level of phosphorylated enzyme. Even in the presence of 8 mM EGTA, trifluoperazine did not accelerate the hydrolysis of phosphorylated enzyme. Similar results were obtained with 1 mM verapamil or TMB-8. Therefore, the reduction of phosphorylated enzyme by the drugs can be attributed to difference in the formation, rather than in the hydrolysis of phosphorylated enzyme.

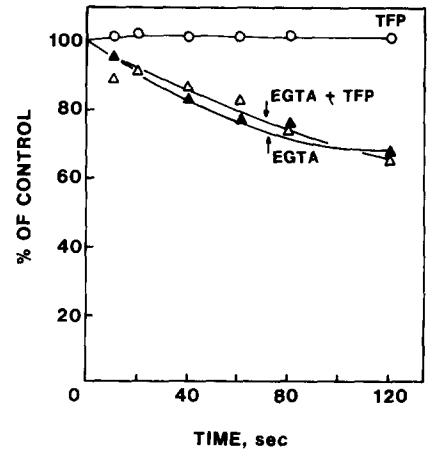

Fig. 4. Plots showing changes in the level of the Ca form of E-P (550 pmol/mg protein) at equilibrium in the presence of 80 μM trifluoperazine (○), 8 mM EGTA (▲) or in combination (△). The chemicals were added 6 min after the initiation of phosphorylation of hog gastric $(H^+ + K^+)$-ATPase, the conditions for which were described in the legend of Fig. 3.

K^+-dependent *p*-nitrophenylphosphatase, copurified with $(H^+ + K^+)$-ATPase, has been often considered as a model reaction for dephosphorylation of the ATPase [21,22]. Fig. 5 shows reciprocal plots of the K^+concentration varying from 0.2 to

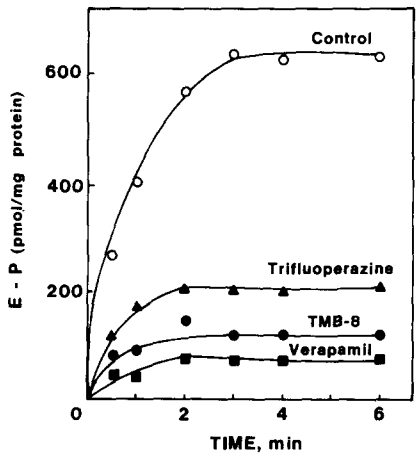

Fig. 3. Time-course profiles for the formation of phosphoenzyme (E-P) in the absence (○) or presence of 80 μM trifluoperazine (▲), 1 mM verapamil (■) or 1 mM TMB-8 (●). The reaction was at 0°C with 5 μM [γ-^{32}P]ATP and 2 mM Ca^{2+}.

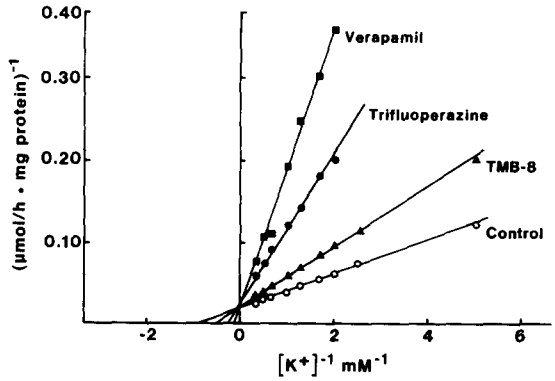

Fig. 5. Lineweaver-Burk plots of the hydrolysis rate of *p*-nitrophenylphosphate by hog gastric membranes versus the concentration of K^+ varying from 0.2 to 3 mM in the absence (○) or presence of 5 μM trifluoperazine (●), 1.4 mM verapamil (■) or 0.5 mM TMB-8 (▲) The V_{max} was 48 μmol/h per mg protein. The incubation was 5 min at 37°C.

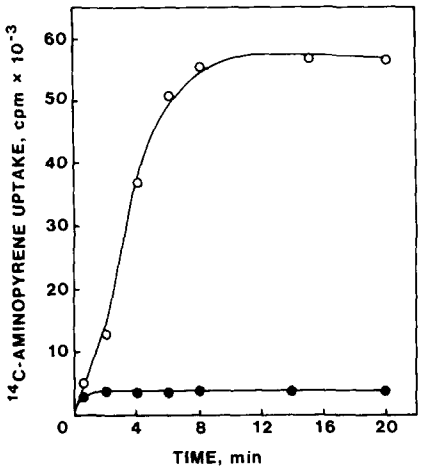

Fig. 6. Time-course profiles of amino[^{14}C]pyrine uptake by hog gastric membranes enriched with ($H^+ + K^+$)-ATPase in the absence (●) or presence (○) of valinomycin (10 μg/ml). The concentration of aminopyrine at the beginning of the reaction was 3 μM in the medium containing 1 mM ATP-Mg^{2+}, 150 mM KCl and 10 mM Pipes, pH 7.0. Incubation was at 22°C.

3 mM versus the hydrolysis rate of *p*-nitrophenyl phosphate observed with or without the drugs. Again, from the Lineweaver-Burk plots, the main effect of the drugs was to increase the K_m for K^+ without affecting the V_{max}; the K_m values for K^+ were 1.0 mM without the drugs, 3.8, 7.5 and 1.8 mM in the presence of 5 μM trifluoperazine, 1.4 mM verapamil or 0.5 mM TMB-8, respectively. The V_{max}, 48 μmol/h per mg protein, remained unchanged. These observations suggest that the drugs compete for K^+-site(s) of both phosphorylated and dephosphorylated forms of the ATPase. Occupation of K^+-site of phosphorylated enzyme by the drugs, however, did not promote dephosphorylation reaction. Rather the drugs inhibited K^+-dependent phosphatase activity. Also the binding of the drugs to dephosphorylated form of the enzyme blocked phosphorylation reaction until the drugs are displaced competitively by a high concentration of K^+, which is consistant with the unchanged V_{max} we observed.

In intact hog gastric membrane vesicles, the operation of ($H^+ + K^+$)-ATPase in the presence of KCl (150 mM), ATP (1 mM) and valinomycin [23,24] results in accumulation of H^+ inside of the vesicles. Fig. 6 shows the extent of amino-

TABLE I

ID_{50} OF TRIFLUOPERAZINE, TMB-8 AND VERAPAMIL FOR AMINO [^{14}C]PYRINE ACCUMULATION AT EQUILIBRIUM IN HOG GASTRIC MEMBRANES VESICLES OR IN ISOLATED RABBIT GASTRIC GLANDS

The membrane vesicles were incubated for 15 min at 22°C and the drugs were added at the beginning of the reaction. The gastric glands were stimulated with 10^{-4} M dibutyryl cAMP and incubated for 45 min at 37°C.

Drugs	Inhibition of amino-[^{14}C]pyrine uptake, ID_{50} (μM)	
	Hog gastric membranes	Rabbit gastric glands
Trifluoperazine	2.0 ± 0.3	7 ± 0.5
TMB-8	10	40 ± 6
Verapamil	9 ± 2	35 ± 7

[^{14}C]pyrine uptake in response to a pH gradient produced by the ATPase [25]. Typically, the distribution ratio of aminopyrine at equilibrium reached about 3000 favoring the intravesicular water space which was found to be 2 μl/mg protein [26]. The concentration of aminopyrine in the incubation media was 3 μM at the beginning of the reaction. Addition of unlabeled aminopyrine up to 30 μM had no effect on the distribution ratio.

The drugs were potent inhibitors of H^+ uptake in the gastric membrane vesicles. The ID_{50} for the aminopyrine accumulation at equilibrium (15 min) was 2 μM for trifluoperazine, 10 μM for verapamil and TMB-8 (Table I). It should be noted that the membrane vesicles were prepared in the presence of 1 mM EGTA and that addition of Ca^{2+} up to 50 μM (in the absence of EGTA) alone or with calmodulin (1 μg/ml) had no noticeable effect on the ID_{50} values of the drugs or the distribution ratio of aminopyrine (data not shown). As further shown in Table I, the ID_{50} values of the drugs for the acid secretion in the gastric glands stimulated by dibutyryl cAMP were quite compatible with those of the drugs to inhibit H^+ uptake in the membrane vesicles.

There was a big discrepancy however, in the potencies of the drugs to inhibit the ATPase in the membranes made freely permeable to cations and to block H^+-uptake in intact membrane vesicles. Therefore, we have measured the ability of the

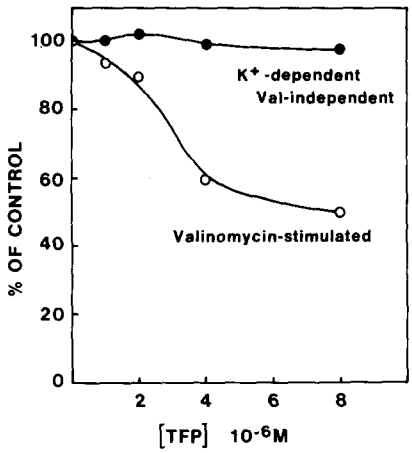

Fig. 7. Effect of trifluoperazine on two types of K^+-dependent ATP hydrolysis observed in hog gastric membrane vesicles developing a pH gradient; the ATP hydrolysis stimulated with valinomycin, 10 μg/ml (○) or observed without valinomycin (●). The reaction was for 5 min at 37°C using the same medium and the membranes as described in the legend of Fig. 6.

drugs to inhibit ($H^+ + K^+$)-ATPase in intact hog membrane vesicles under the conditions to develop a pH gradient. Two types of ATP-hydrolysis in these membrane vesicles were observed; one is K^+-dependent ATP-hydrolysis, 47 μmol/h per mg protein, in the absence of valinomycin probably representing a population of the vesicles leaky to K^+, and the other is the valinomycin-stimulated ATP-hydrolysis amounting to 52 μmol/h per mg protein additionally. Trifluoperazine at 4 μM inhibited almost 40% of the valinomycin-stimulated ATPase activity, but had no effect on the valinomycin-independent K^+-dependent ATPase activity (Fig. 7). Verapamil and TMB-8 at 15 μM also reduced selectively the valinomycin-stimulated ATP hydrolysis by about 30%. Uptake of H^+ as measured by aminopyrine accumulation occurs only with valinomycin in these membrane vesicles. Furthermore, we observed that 40% inhibition of valinomycin-stimulated ATPase activity led to more than 90% reduction in aminopyrine accumulation at equilibrium. The difference in the degrees of inhibition of the ATPase and aminopyrine accumulation could be either due to a considerable portion of H^+ pumping activity of the ATPase being nullified by H^+ leak in the membrane vesicles and/or due to displacement of aminopyrine by the drugs. The extents of aminopyrine displacement by the drugs are not likely to be significant, however, since various other weak bases having no effect on the ATPase at the concentrations of 30 μM or higher did not interfere aminopyrine accumulation. Such bases include aminopyrine, pyridine, piperidine, phenothiazine and alipathic tertiary amines. The improved potency of the drugs in the membrane vesicles developing a pH gradient (acidic inside) can be attributed to the accumulation of the drugs as a weak base inside of the vesicles and subsequent interaction with the luminal K^+-site of the

TABLE II

DOSES OF CHLORPROMAZINE AND OTHER LIPOPHILIC AMINES TO INHIBIT 50% ($H^+ + K^+$)-ATPASE AND AMINO [^{14}C]PYRINE ACCUMULATION AT EQUILIBRIUM IN HOG GASTRIC MEMBRANES OR IN RABBIT GASTRIC GLANDS

The value of ID_{50} was obtained from a typical dose-response curve consisting of six points. Standard errors were found less than 25%.

	ID_{50} (M)		
	Inhibition of gastric ($H^+ + K^+$)-ATPase [a]	Inhibition of amino[^{14}C]pyrine uptake	
		Hog gastric membranes	Rabbit gastric glands
Chlorpromazine	$5 \cdot 10^{-5}$	$4 \cdot 10^{-7}$	$1 \cdot 10^{-6}$
Azacosterol	$1 \cdot 10^{-5}$	$8 \cdot 10^{-6}$	$2.5 \cdot 10^{-5}$
1-[3-(Dimethylamino)propyl]-indole	$2.5 \cdot 10^{-3}$	$3 \cdot 10^{-6}$	$9 \cdot 10^{-5}$
Plasmocid	$1.5 \cdot 10^{-4}$	$2 \cdot 10^{-6}$	$2.5 \cdot 10^{-5}$
Iprindole	$6 \cdot 10^{-5}$	$7 \cdot 10^{-7}$	$2.5 \cdot 10^{-6}$

[a] The ATPase assays were carried out utilizing hog gastric membranes freely permeable to K^+ and in the presence of 20 mM KCl.

ATPase, which is accessible from the inside of the vesicle.

We have additionally tested chlorpromazine and other lipophilic amine drugs for their effect on gastric $(H^+ + K^+)$-ATPase, and on amino [^{14}C] pyrine uptake in the gastric membranes and in isolated rabbit gastric glands (Table II). Chlorpromazine, plasmocid, 1-[3-dimethylamino)-propyl]indole, azacosterol and iprindole inhibited $(H^+ + K^+)$-ATPase and blocked H^+ accumulation in the membrane vesicles and in the glands as measured by aminopyrine distribution.

Discussion

In this study, trifluoperazine, verapamil and TMB-8 have been shown to inhibit H^+-uptake in isolated hog gastric membrane vesicles by blocking $(H^+ + K^+)$-ATPase. Kinetic studies indicated competititve inhibition of the ATPase by the drugs with respect to K^+. There are two K^+-sites known for the gastric ATPase [15]. One is of low affinity located on the cytoplasmic surface of the ATPase and inhibitory to phosphorylation of the enzyme. The other is a high affinity site (K_m) $\leq$ 1 mM) on the luminal face of the enzyme and is stimulatory to dephosphorylation of the phosphoenzyme. It appeared from this study that the primary effect of trifluoperazine, verapamil and TMB-8 comes from their interaction with the luminal K^+-site of the ATPase, since the drugs primarily competed for the K^+-site of a low K_m, 0.43 mM, and their inhibitory potency increased as they were accumulated in the acidic luminal side of the membranes as a weak base.

The drug also inhibited K^+-dependent *p*-nitrophenylphosphatase activity of the ATPase again in competition with K^+. The K^+-site of the phosphatase is likely to be accessible from the cytoplasmic side of the enzyme as the case with $(Na^+ + K^+)$-ATPase [27,28]. Nevertheless, the site is of relatively high affinity for K^+ ($K_m \sim 1$ mM) and is involved in the dephosphorylation reaction. Considering these factors, the K^+-site of the phosphatase is more comparable to the luminal site rather than the cytoplasmic site of the ATPase. The competititve inhibition of the phosphatase activity by the drugs, therefore, is consistent with the proposed interaction of the drugs with the luminal K^+-site of the ATPase.

The following scheme may represent a simplified version of the catalytic reactions of gastric $(H^+ + K^+)$-ATPase in analogy with $(Na^+ + K^+)$-ATPase [15,22]:

$$E_1 + ATP\text{-}Mg^{2+} \xrightarrow{H^+} E_1\text{-}P \leftrightarrow E_2\text{-}P \xrightarrow{K^+} E_2\text{-}P\cdot K$$

$$E_2\text{-}P\cdot K \leftrightarrow E(K) + P_i$$

$$E(K) \leftrightarrow E_1 + K^+$$

In this scheme, luminal K^+ binds the E_2-P form, one of two major conformational states of the ATPase. Since the drugs compete with K^+, it is probable that the drugs also favor the E_2 form of the enzyme. The reduced level of phosphoenzyme we observed in the presence of the drugs further suggests that the drug-enzyme complex could not undergo the conformational change to E_1.

Of considerable interest is what structural features of the drugs are responsible for their interaction with the K^+-site of the ATPase. A tertiary amine is the only common functional group of the drugs and upon protonation is likely to interact with K^+-site, since NH_4^+ substitutes for K^+ in the ATPase. This idea was further supported by our observations that various lipophilic amines possessed the ability to inhibit ATPase and block the acid secretion in the glands (Table II). In addition, several investigators recently reported gastric antisecretory activity of various amine drugs in vivo or in vitro. Columbo et al. [29] reported gastric antisecretory activity of 4-(*p*-sulfamylbenzoyl)-*N*-methylpiperidine in the rat. Ray et al. [30] showed gastric antisecretory effect of polyamines such as spermine and spermidine on isolated frog gastric mucosa and further noted the disappearance of the activity on increasing the K^+ concentration of the medium. Bristol et al. [31] reported the antisecretory activity in the rat and dog of a series of xanthine analogs substituted with various amines.

Another interesting structural aspect of the drugs is that their potency to inhibit $(H^+ + K^+)$-ATPase was apparently influenced by their hydrophobic moiety. For instance, the phenothiazine derivatives were 20- to 40-times more potent than the indole or quinoline drugs. This observation points out the importance of certain hydrophobic

interactions of the drugs with the ATPase affecting their apparent affinity for the K^+-site.

It is difficult to predict whether trifluoperazine, verapamil or TMB-8 would be also effective as a K^+-blocker in other animal cells. Influencing factors are the affinity of the drugs for a given K^+-site, the concentration of K^+ near the site and the presence or absence of a pH gradient which affects the effective concentration of the drugs. Several amine drugs have been known as K^+-channel blockers: tetraethylammonium and aminopyridine in squid axon membranes [32], quinine and quinidine in β-cells from mouse pancreatic islets of Langerhans [33] and phencyclidines in rat brain synaptosomes [34]. It is worth mentioning some known calmodulin-independent actions of trifluoperazine or chlorpromazine such as disruption of ATP levels in mitochondria [10] and lymphocytes [11]. Verapamil also has been reported to affect heart mitochondrial respiration only in a low-K^+ (5 mM) medium, but not in a high-K^+ (150 mM) one [35]. Certainly, the possibility exists that these drugs could alter cellular metabolism by their K^+- or Ca^{2+}-antagonistic action, depending on concentration and cell type.

References

1 Levin, R.M. and Weiss, B. (1978) Adv. Cyclic Nucleotide Res. 9, 285–303
2 Wolff, D.J. and Brostrom, C.O. (1979) Adv. Cyclic Nucleotide Res. 11, 27
3 Wolff, D.J. and Brostrom, C.O. (1981) Biochem. Pharmacol. 30, 1395–1405
4 Rahwan, R.G., Piascik, M.F. and Witiak, D.T. (1979) Can. J. Physiol. Pharmacol. 57, 443–460
5 Fleckenstein, A., Nakayama, K., Fleckenstein-Grun, G. and Byon, Y.K. (1976) Coronary Angiography and Angina Pectoris (Lichter, P.L., ed.), pp. 297–315, Publ. Sci. Group, Action, MA
6 Chiou, C.Y. and Malagodi, M.H. (1975) Br. J. Pharmacol. 53, 279–285
7 Rasmussen, H. and Goodman, B.P. (1977) Physiol. Rev. 57, 421–509
8 Raphael, N., Ekbead, E.B.M. and Machen, T.E. (1982) Fed. Proc. 41, Abstr. 4155
9 Spenny, J.G., Sack, J., Malinowska, D.H., Rabon, E. and Sachs, G. (1982) Fed. Proc. 41, Abstr. 7181
10 Ruben, L. and Rasmussen, H. (1981) Biochim. Biophys. Acta 637, 415–422
11 Corps, A.N., Hesketh, T.R. and Metcalfe, J.C. (1982) FEBS Lett. 138, 280–284
12 Chew, C.S., Hersey, S.J., Sachs, G. and Berglindh, T. (1980) Am. J. Physiol. 238, 312–320
13 Berglindh, T., Dibona, D.R., Ito, S. and Sachs, G. (1980) Am. J. Physiol. 238, G165–G176
14 Saccomani, G., Stewart, H.B., Shaw, D., Lewin, M. and Sachs, G. (1977) Biochim. Biophys. Acta 465, 311–330
15 Stewart, B., Wallmark, B. and Sachs, G (1981) J. Biol. Chem. 256, 2682–2690
16 Berglindh, T. and Obrink, K.J. (1976) Acta Physiol. Scand. 96, 150–159
17 Tsai, C.M., Chen, K-Y. and Canellakis, E.S. (1975) Biochim. Biophys. Acta 401, 196–212
18 Berglindh, T. (1977) Biochim. Biophys. Acta 464, 217–233
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 165–175
20 Mendlein, J. and Sachs, G. (1982) Fed. Proc. 41, Abstr. 6431
21 Forte, J.G., Ganser, A.L. and Tanisawa, A.S. (1974) Ann. N.Y. Acad. Sci. 242, 255–267
22 Sachs, G., Spenney, J.G. and Lewin, M. (1978) Physiol. Rev. 58, 106–173
23 Chang, H., Saccomani, G., Rabon, E., Schackmann, R. and Sachs, G. (1977) Biochim. Biophys. Acta 464, 313–327
24 Lee, H.-C., Breitbart, H., Berman, M. and Forte, J.G. (1979) Biochim. Biophys. Acta 464, 313–327
25 Rabon, E., Chang, H. and Sachs, G. (1978) Biochemistry 17, 3345–3353
26 Sachs, G., Chang, H.H., Rabon, E., Schackman, R., Lewin, M. and Saccomani, G. (1976) J. Biol. Chem. 251, 7690–7698
27 Blostein, R., Pershadsingh, H.A., Drapeau, P. and Chu, L. (1979) Na,K-ATPase: Structure and Kinetics (Skou, J.C. and Norby, J.G., eds.), pp. 233–245, Academic Press, London
28 Robinson, J.D. and Mercer, R.W. (1981) J. Bioenerg. Biomembrane 13, 205–218
29 Colombo, R., Pinelli, A. and Muserra-Grandi, D. (1981) Pharmacol. Res. Commun. 13, 525–534
30 Ray, T.K., Nandi, J., Pidhorodeckyj, N. and Meng-Ai, A. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 1448–1452
31 Bristol, J.A., Gold, E.H., Gross, I., Lorey, R.G. and Long, J.F. (1981) J. Med. Chem. 24, 1010–1013
32 Yeh, J.Z., Oxford, G.S., Wu, C.H. and Narahashi, T. (1976) J. Gen. Physiol. 68, 519–535
33 Atwater, I., Dawson, C.M., Ribalet, B. and Rojas, E. (1979) J. Physiol. 288, 575–588
34 Albuquerque, E.X., Aguayo, L.G.,,Warnick, J.E., Weinstein, H., Glick, S.D., Maayani, S., Ichowicz, R.K. and Blaustein, M.P. (1981) Proc. Natl. Acad. Sci. U.S.A. 78, 7792–7796
35 Vaghy, P.L., Matlib, M.A., Szerkeres, L. and Schwartz, A. (1981) Biochem. Pharmacol. 30, 2603–2610