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Polyamines are inhibitors of gastric acid secretion

(chambered mucosa/gastric microsomes/H^+,K^+-ATPase/vesicular H^+ transport)

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ABSTRACT **The naturally occurring organic polycations such as spermine and spermidine inhibit histamine-stimulated gastric acid secretion by bullfrog gastric mucosa *in vitro*; spermine is much more potent than spermidine. Unlike the H_2 receptor antagonists, the polyamines are completely ineffective from the nutrient side and are effective only from the secretory side of the chambered mucosa. The polyamine effects could be reversed by increasing K^+ concentration in the secretory solution. Studies with isolated gastric microsomal vesicles demonstrate that the polyamines do not inhibit the gastric H^+,K^+-ATPase but greatly decrease the ATPase-mediated uptake of H^+ under appropriate conditions. For the latter effects the presence of polyamine within the vesicle interior was found to be essential. Our data strongly suggest an uncoupling of the gastric H^+,K^+-ATPase system by the polyamines. The therapeutic potential of these and similar compounds in the treatment of hyperacidity and peptic ulcer is discussed.**

A recent report (1) on the mechanism of thiocyanate inhibition of gastric acid secretion by bullfrog gastric mucosa *in vitro* states that SCN^- competes with K^+ at or near the apical membrane of the parietal cells for some steps leading to H^+ transport. Subsequent studies with isolated gastric microsomal vesicles enriched in H^+,K^+-ATPase, which has been identified to be the enzymatic mechanism for H^+ transport (2), demonstrated that SCN^- uncouples the gastric ATPase-mediated K^+/H^+ exchange without inhibiting the hydrolysis of ATP (ref. 3; unpublished data). This uncoupling effect of SCN^- may occur by virtue of its binding with a low-affinity KCl binding site, presumably the Cl^- site, within the domain of the gastric H^+,K^+-ATPase. The low-affinity KCl site (S_{50} = 19 mM)* accessible from the lumen may be responsible for the vectorial transport of H^+ across the apical membrane of the acid-secreting cells. In an effort to find further support of this hypothesis, we wanted to find some cations that would compete with the luminal low-affinity K^+ site without entering into the cells and thus uncouple the gastric H^+ transport mechanism. It appeared likely that the organic polycations such as spermine, spermidine, and putrescine may be candidates for such effects.

In the present study we tested the effects of the polyamines on *in vitro* H^+ transport by bullfrog gastric mucosa as well as by the isolated gastric microsomal vesicles. Our data demonstrated that the diamines were without much effect but both spermine and spermidine did uncouple the gastric H^+ transport mechanism and that the inhibition of H^+ secretion could be reversed by increasing the K^+ concentration in the secretory solution of the chambered mucosa. Furthermore, the uncoupling effect of the polyamines was only exerted when they were present in the secretory solution of the chambered mucosa or interior of the microsomal vesicles. Spermine was found to be a more effective inhibitor of H^+ transport than spermidine, both in the chamber situation and in the microsomal vesicles.

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EXPERIMENTAL PROCEDURE

Transport Studies with Chambered Bullfrog Gastric Mucosa. All experiments were carried out with gastric mucosa from bullfrogs (*Rana catesbeiana*). After the frogs had been pithed, fundic mucosae were carefully separated from the submucosae and mounted over one end of a plastic tube (13 × 100 mm) with the mucosal surface facing out. The area of the mounted mucosa was 1.3 cm^2. The bathing solutions were bubbled continuously with 95% O_2/5% CO_2.

The normal nutrient solution (1) had the following composition (in mM): NaCl, 87; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 1; KH_2PO_4, 1; $NaHCO_3$, 18; and glucose, 11. The secretory solution was 104 mM NaCl. In some experiments, as indicated, various amounts of K^+ were used in the secretory solution, an equivalent amount of Na^+ being taken out to maintain the final concentration of cation at 104 mM. For the experiments in which no K^+ was in the nutrient medium, an equivalent amount of NaCl was substituted for K^+. It took about 6 hr for the spontaneously secreting mucosa maintained in 0 K^+ medium to come to the resting (near-zero secretion) level, as reported (1).

All experiments were conducted under open-circuit conditions at room temperature. The mucosal solutions were collected at 15-min intervals and placed in thoroughly washed plastic vials. The K^+ content of the secretory medium was determined by atomic absorption (model 360 spectrophotometer, Perkin–Elmer). The H^+ secretion was quantitated by titration with 1 mM NaOH to pH 6.5 during gassing with 100% N_2.

The unpaired Student *t* test was used to compare secretory data obtained in test conditions with initial steady-state values, and the differences were regarded as significant when $P < 0.05$. The results are expressed as means ± SEM.

Isolation of Gastric Microsomes. Fresh pig stomachs were purchased from the local slaughterhouse. The gastric microsomal membranes were harvested as described (4). All procedures were carried out at 0–4°C. Briefly, the fundic mucosa from the pig was desquamated and scraped (5) to collect the oxyntic cell-enriched fractions. The mucosal scraping was homogenized gently in 250 mM sucrose/0.2 mM EDTA/0.2 mM Pipes, pH 6.8, by using a Dounce homogenizer with a loose pestle. The homogenate was centrifuged at 8000 × *g* for 5 min. The process was repeated three times. All the supernatants were pooled and layered over 40 ml of 37% sucrose in 84-ml-capacity screw-cap tubes and centrifuged at 100,000 × *g* for 5 hr in a type 35 Beckman angular rotor. The microsomal membrane bands appeared at the interface of the soluble supernatant and 37% sucrose. The microsomal bands were collected, diluted with homogenizing medium, and centrifuged at 100,000 × *g* for 90 min. The pellet was suspended in the homogenizing medium with a protein concentration of 0.5 mg/ml and used in our study. The proteins were assayed by the Lowry procedure (6).

*S_{50} is the concentration that yields 50% of the V_{max} and was determined from the Hill plot of the data on the rate of vesicular H^+ transport with increasing K^+ in the medium (unpublished data).

Assay of ATPase. The ATPase was assayed as described (7). Briefly, the incubation mixture contained, in a total volume of 1 ml, 50 μmol of Pipes pH 6.8 buffer, 1 μmol of $MgCl_2$, 2 μmol of Tris ATP, and 20 μg of membrane protein with or without 150 μmol of KCl in presence and absence of 10 μM valinomycin. After 10-min incubation at 21°C, the reactions were stopped by addition of 1 ml of 12% (wt/vol) CCl_3COOH. The P_i was measured by the procedure of Sanui (8).

Study of Vesicular H^+ Uptake. Vesicular accumulation of H^+ was measured at room temperature according to Lee and Forte (9) and as described (7). The method uses the fact that change in fluorescence intensity (quenching) of 9-aminoacridine or acridine orange is proportional to the amount of dye taken up by the microsomes. The amount of dye taken up is a sensitive measure of intravesicular H^+ concentration. Wavelengths used were 493 and 530 nm (excitation and emission) for acridine orange in the Aminco–Bowman spectrofluorometer.

RESULTS

Effects of Polyamines on Gastric H^+ and K^+ Transport by Bullfrog Gastric Mucosa *In Vitro*. Addition of polyamines to either the nutrient or the secretory solution of a histamine-stimulated bullfrog gastric mucosa had different effects on H^+ transport. Spermine at 0.5 and 1.0 mM did not have any appreciable effect from the nutrient side (Fig. 1 *Lower*). However, when added into the secretory solution, spermine inhibited H^+ transport at a concentration of 0.25 mM, and the inhibition increased with increasing concentrations of spermine (Fig. 1 *Upper*). The inhibition of H^+ transport by spermine on the secretory side was completely reversible by increasing the K^+ concentration in the secretory solution. Similar reversibility, by high K^+, of SCN^- inhibition of gastric H^+ transport has been reported (1, 10) and an antagonism between K^+ and SCN^- at the secretory membrane for some final critical steps leading to H^+ transport has been demonstrated (1).

Spermidine also inhibited gastric H^+ transport when added to the secretory solution (Fig. 2) but not when in the nutrient solution (data not shown). However, the inhibitory effects of spermidine were much lower than those of spermine. The spermidine effects also could be reversed by increasing the K^+ concentration in the secretory solution. The effects of different polyamines on the steady-state level of H^+ transport by histamine-stimulated gastric mucosa are compared in Table 1. The diamines at 0.5 mM were totally ineffective in inhibiting gastric acid secretion.

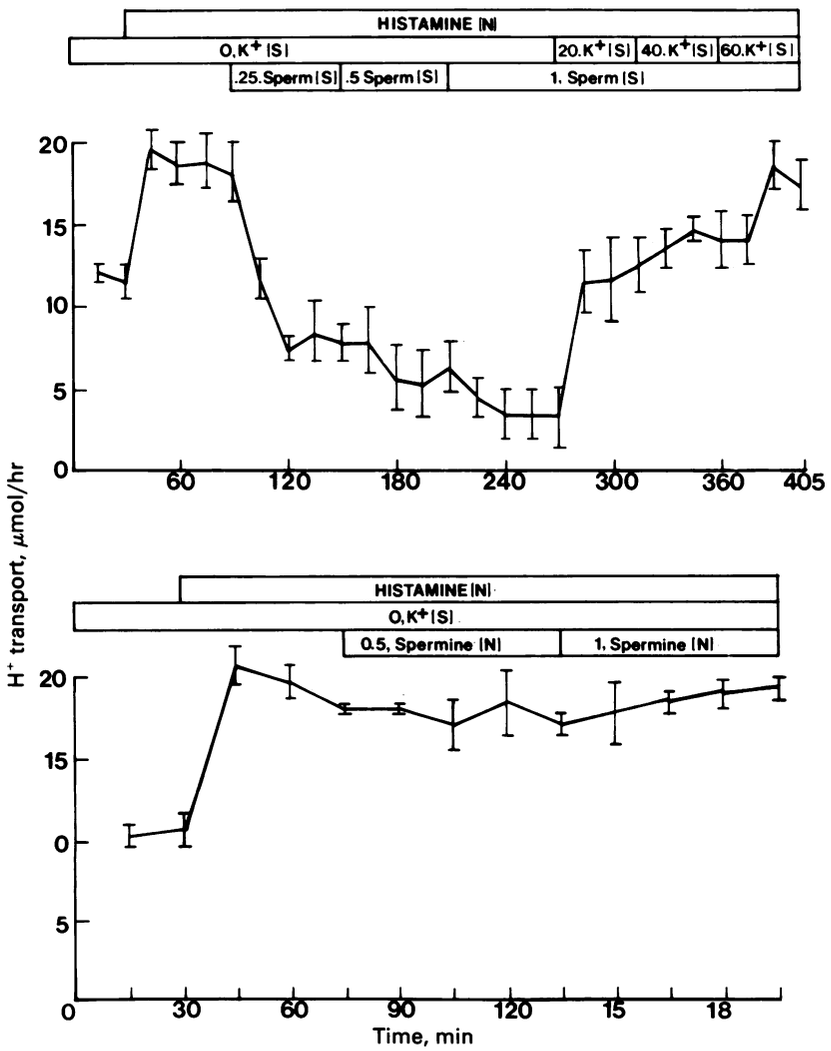

FIG. 1. (*Upper*) Effects of incorporation of different concentrations (mM) of spermine (Sperm) and K^+ in the secretory solution on H^+ transport by histamine-stimulated bullfrog gastric mucosa *in vitro*. N, nutrient solution; S, secretory solution. Values are mean ± SEM (n = 6). (*Lower*) Effects of different concentrations (mM) of spermine in the nutrient solution on H^+ transport by histamine-stimulated bullfrog gastric mucosa *in vitro*. Values are mean ± SEM (n = 6).

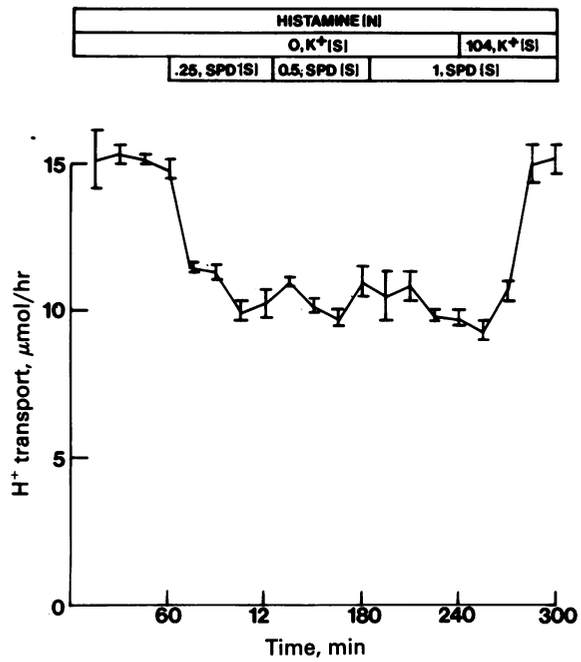

FIG. 2. Effects of different concentrations (mM) of spermidine (SPD) on H^+ transport and the reversibility of the spermidine effect by K^+. N, nutrient solution; S, secretory solution. Data are mean ± SEM (n = 3).

It has been previously demonstrated (1, 11) that the presence of K^+ in either the nutrient or secretory bathing medium of the chambered mucosa is absolutely necessary for gastric acid secretion. When the mucosa was maintained in a K^+-free medium for 2–3 hr, the histamine-stimulated acid secretion became significantly low but was tripled after incorporation of 10 mM K^+ into the secretory solution (Fig. 3). However, the presence of 1 mM spermine with 10 mM K^+ in the secretory solution decreased the rate of H^+ transport to nearly zero within 30 min. This inhibitory effect could be reversed by increasing the secretory-side K^+, demonstrating an antagonism between K^+ and

Table 1. Effects of spermine, spermidine, putrescine, and propanediamine on histamine-stimulated steady-state H^+ secretory rate

Drug in secretory solution	Rate of H^+ transport, % of control	n
None	100	10
Spermine	28 ± 6	6
Spermidine	55 ± 2	6
Putrescine	95 ± 10	3
Propanediamine	92 ± 8	3

The H^+ secretory rates are the steady-state values before and after addition of the drugs (0.5 mM) attained by the histamine-stimulated mucosa. Values are mean ± SEM.

spermine at or near the secretory membrane for some step or steps leading to gastric H^+ transport. A similar antagonism between secretory K^+ and SCN^- for gastric acid secretion has been reported (1).

The relationship between H^+ and K^+ transport in the presence of 1 mM spermine in the secretory solution is shown in Fig. 4. Although spermine inhibited H^+ transport in spontaneously secreting mucosa, it had no effect on K^+ transport under similar conditions. However, stimulation by histamine with spermine on the mucosal side caused a transient increase in K^+ transport which returns to the prestimulated control level within 90 min; the H^+ transport remaining nearly unaltered under these conditions. Removal of spermine from the secretory solution with histamine on the nutrient side did not appreciably change either the H^+ or K^+ transport rates, possibly because of strong binding of some residual spermine molecules with the secretory membrane H^+ transport mechanism. Addition of 40 mM K^+ to the secretory solution without added spermine reversed the H^+ transport within 30 min. Unlike the effects observed after removal of the secretory spermine from the histamine-stimulated mucosa, SCN^- inhibition of H^+ transport could be fully reversed by simply removing SCN^- from the bathing medium (1).

Effects of Spermine and Spermidine on Gastric Microsomal H^+,K^+-ATPase and Vesicular H^+ Uptake. Gastric microsomal

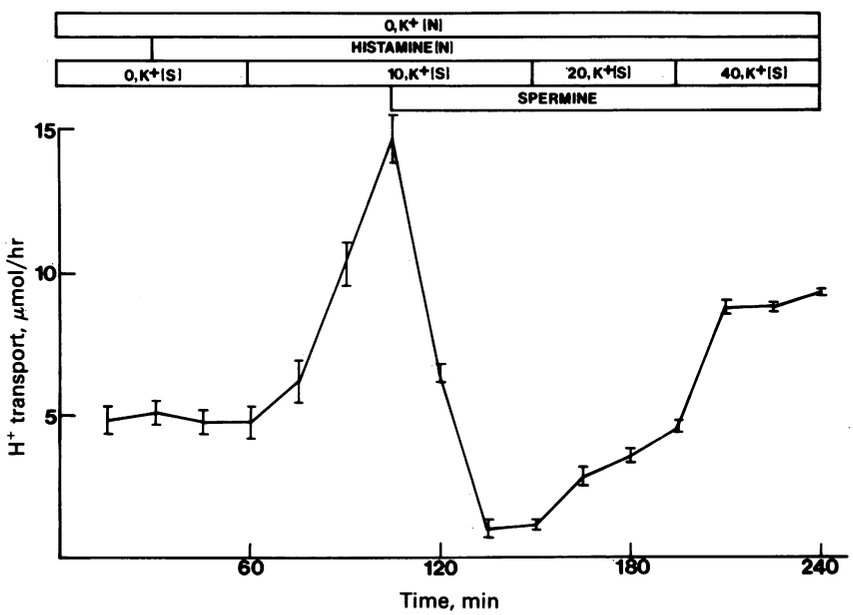

FIG. 3. Stimulation of H^+ transport by secretory-side K^+ in mucosa maintained in a K^+-free (0 K^+) medium and the reversal of spermine inhibition by higher concentrations (mM) of secretory-side K^+. The mucosae were maintained in K^+-free medium for about 3 hr. N, nutrient solution; S, secretory solution. Data are mean ± SEM (n = 5).

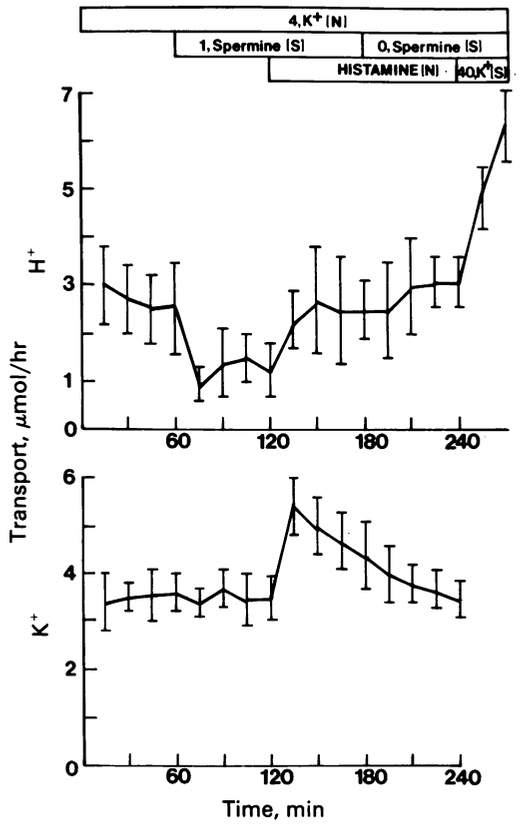

FIG. 4. Effects of sequential treatment with spermine and histamine on H^+ (*Upper*) and K^+ (*Lower*) transport by the chambered mucosa. N, nutrient solution; S, secretory solution. Data are mean ± SEM (n = 8).

vesicles derived primarily from the apical and tubulovesicular membranes of the parietal cells are highly enriched in H^+,K^+-ATPase which has been identified as the enzymatic mechanism for the transport of H^+ in exchange for K^+ (12). The data in Fig. 5 show the effects of spermine on vesicular H^+ uptake mediated by the gastric H^+,K^+-ATPase system. Spermine at 0.5 mM did not have any appreciable effect on H^+ uptake by KCl-nonequilibrated vesicles, in which the intravesicular requirement of K^+ for H^+ transport process is met by the addition of valinomycin. However, when the vesicles were preequilibrated in 150 mM KCl with and without 0.5 mM spermine at 0–4°C for 48 hr, the H^+ uptake by the vesicles containing spermine was totally obliterated. Soon after the rapid accumulation of H^+ in the control microsomes by the K^+/H^+ exchange mechanism, depletion of the intravesicular K^+ occurred. At this point, no more H^+ was pumped in, because of unavailability of exchangeable intravesicular K^+; hence, a net leakage of H^+ was observed. However, when valinomycin was added at this stage to allow K^+ in, a net accumulation of H^+ by the vesicles again was observed. The data suggest that microsomal vesicles are relatively impermeable to spermine and the spermine effects are exerted from the vesicle interior. Poor diffusion of spermine across gastric microsomes is also demonstrated by the data; no effects of spermine were observed when added together with ATP to the KCl-preequilibrated vesicles.

In separate experiments (data not shown) we tested the effects of spermidine on vesicular H^+ transport. Similar to spermine, spermidine at concentrations up to 1.0 mM had no appreciable effect on valinomycin-stimulated acridine orange uptake. Spermidine also substantially decreased (50–70%) the transport of H^+ inside the vesicles preloaded with spermidine at concentrations of 0.1–0.5 mM. Furthermore, like spermine, spermidine did not have any effect on vesicular H^+ uptake when added together with ATP to the KCl-preequilibrated vesicles.

Table 2. Effects of spermine on H^+,K^+-ATPase activity associated with isolated pig gastric microsomal vesicles

Conditions	ATPase activity, μmol/mg protein per hr		
	Mg^{2+} alone	With K^+	With K^+ and valinomycin
Nonpreequilibrated vesicles			
Control	2.32 ± 0.14	7.56 ± 0.48	13.12 ± 0.20
Plus 0.5 mM spermine	2.35 ± 0.28	6.49 ± 0.19	12.71 ± 0.06
Preequilibrated vesicles			
Control	3.11 ± 0.18	5.72 ± 0.37	8.32 ± 0.06
Plus 0.25 mM spermine	3.22 ± 0.47	5.82 ± 0.11	8.17 ± 0.04
Plus 0.5 mM spermine	2.09 ± 0.08	7.19 ± 0.31	8.81 ± 0.36

For the nonpreequilibrated vesicles, spermine was incorporated into the assay medium during ATPase measurement. For the preequilibrated vesicles, the microsomes were preincubated with either 150 mM KCl (control) or KCl plus the desired concentrations of spermine in 5 mM Pipes/0.2 mM EDTA, pH 6.8, at 0–4°C for 48 hr. The ATPase activity was measured at room temperature. Data are mean ± SEM (n = 4).

The effects of spermine on H^+,K^+-ATPase activity of gastric microsomes were studied under the condition of vesicular H^+ uptake as described in Fig. 5. No appreciable effect of spermine was observed on the gastric K^+-stimulated ATPase activity both in presence and absence of valinomycin (Table 2). Like spermine, spermidine also did not have any appreciable effect on gastric H^+,K^+-ATPase activity under similar conditions (unpublished data).

DISCUSSION

Our data reveal that spermine and spermidine are effective inhibitors of gastric acid secretion by histamine-stimulated bullfrog gastric mucosa, spermine being more potent than spermidine. The diamines are virtually ineffective at comparable dose levels. The data (Fig. 1) also demonstrate that the antisecretory effects of polyamines are manifested only from the luminal side of the chambered mucosa, suggesting that the acid-secreting cells are relatively impermeable to the highly charged polyamines and that the effects are exerted at the secretory membrane accessible from the luminal side. This idea is consistent with the ready reversibility of the polyamine effects by high secretory-side K^+ (Fig. 1). Furthermore, the specificity of K^+ in the reversal of spermine effects (Fig. 3) suggests interaction of the polyamine with some specific luminal K^+ sites essential for the H^+ transport mechanism.

Effects of spermine on K^+-stimulated ATPase-mediated uptake of H^+ by isolated gastric microsomal vesicles in the valinomycin/K^+ system (Fig. 5) demonstrate that the agent is ineffective at up to 0.5 mM. However, in the vesicles preequilibrated with spermine and KCl, the inhibitory effects of spermine on vesicular H^+ transport is observed at concentrations comparable to those used in the chamber study (Fig. 1). Thus, these data are consistent with our conclusion (see above) that gastric membranes are relatively impermeable to spermine and the site of action of the polyamine is located within the vesicle interior. This conclusion is further supported by our data showing that, when spermine is added together with ATP to the KCl-preequilibrated vesicles, the vesicular uptake of acridine orange is nearly the same as that without spermine (Fig. 5).

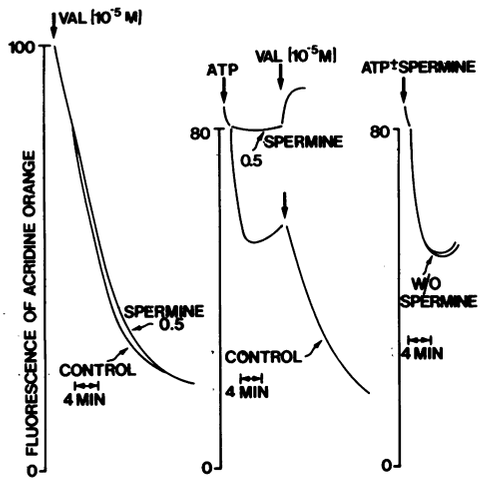

FIG. 5. (*Left*) Effects of spermine (0.5 mM) on valinomycin (VAL)-induced acridine orange uptake by isolated gastric microsomal vesicles. The 2-ml incubation mixture was 20 mM Pipes, pH 6.8/0.5 mM EDTA/1 mM Mg^{2+}/150 mM KCl/1 mM ATP/0.01 mM acridine orange containing 100 μg of microsomal protein in the presence and absence of 0.5 mM spermine. The reaction was started by adding 10 μM valinomycin and was carried out at room temperature. (*Center*) Effects of preequilibration of gastric microsomal vesicles with 150 mM KCl in the presence and absence of 0.5 mM spermine on ATP-induced acridine orange uptake. The vesicles were preequilibrated for 48 hr at 0–4°C. The composition of the incubation medium was as in *Left*. The reactions were first initiated with 1 mM ATP and then valinomycin was added later as indicated by the arrow. (*Right*) Effects of spermine (0.5 mM) on ATP-induced acridine orange uptake by vesicles preequilibrated with 150 mM KCl. The experimental conditions were as in *Center*.

The gastric K^+-stimulated ATPase, which has been identified as the enzymatic mechanism for gastric K^+/H^+ exchange pump (2), is not inhibited by the polyamines (Table 2) under similar conditions of microsomal H^+ transport. Therefore, the antisecretory effects of polyamines appear to be exerted by some kind of uncoupling of the gastric K^+-stimulated ATPase system.

Resting (unstimulated) gastric mucosa transports K^+ from the cell into the lumen at a slow steady rate (13) and the process has been characterized as a passive one (1). Upon stimulation with secretagogues, the mucosa quickly responds with a K^+ transient that has been suggested to be due to a combined effect of an enhanced transport of K^+ from the cell interior into the lumen and recycling of the luminal K^+ back into the cell in exchange for H^+ (1, 13). Our previous studies (1, 10, 13) also demonstrated that inhibition of H^+ transport in secretagogue-stimulated mucosa by addition of *p*-chloromercuribenzenesulfonic acid into the secretory-side solution results in a sustained increase in K^+ transport due to inhibition of the K^+/H^+ exchange pump.

The present data on the effects of mucosal spermine in unstimulated mucosa maintained in a regular nutrient medium demonstrate that spermine does not interfere with the passive diffusion of K^+ (Fig. 4). Also, under conditions of histamine stimulation and with spermine in the secretory-side solution, the mucosa shows a K^+ transport (Fig. 4) characteristic of stimulated control mucosa (11, 14). In view of the fact that spermine does not inhibit gastric H^+,K^+-ATPase (Table 2) but inhibits H^+ transport (Figs. 5 and 1) the data (Fig. 4) suggest that, unlike P-chloromercuribenzenesulfonate (10, 13) as discussed above, spermine does not affect the recycling of K^+ although it inhibits H^+ transport. Therefore, the data (Table 2; Fig. 5) strongly suggest that spermine uncouples the K^+/H^+ exchange pump in the secretory membrane in such a way that the vectorial transport of H^+ ceases or is altered although the transport and recycling of K^+ remain uninterrupted. This uncoupling effect of spermine appears to be exerted by way of binding with a luminal K^+ site.

Because spermine is more potent in inhibiting gastric acid secretion than its lower homologues such as spermidine and putrescine, it is highly likely that the higher homologues of spermine or some of their derivatives may be still more effective inhibitors. Furthermore, because these agents do not diffuse through gastric membranes and, unlike H_2 antagonists, are effective only from the secretory (lumen) side of the tissue, it appears that this class of compounds would have a therapeutic potential ideal for the treatment of hyperacidity and peptic ulcer. Also, other parts of the gastro-intestinal tract would be expected to be poorly permeable to these highly charged organic cations as well. Although the polyamines are known to be important functional components of the living cells (14), the higher synthetic homologues or their derivatives may prove to be toxic if administered into the bloodstream. However, due to the expected poor absorbability of those compounds from the gastrointestinal tract, the risk of systemic toxicity would be greatly minimized.

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