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Effects of a Naturally Occurring Polyamine on Acid Secretion by Isolated Gastric Mucosa (41765)

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Abstract. Determination of the effects of spermine on acid secretion by isolated rabbit gastric mucosa shows paradoxical responses at neutral luminal pH. Initial inhibition of acid secretion was followed by a return to near basal rates. However, measurement of mucosal and serosal rates of CO_2 release indicated that spermine causes prolonged inhibition of acid secretion. Similar prolonged inhibition is seen with mucosa exposed to an acidic luminal pH. The inhibitory effect of spermine is reversed by the addition of K^+ to the mucosal side, suggesting spermine interferes with a K^+ site at the secretory membrane. Serosal addition of spermine is without effect. The apparent acid secretory rebound phenomenon observed after the addition of spermine is most likely related to formation of H^+ in the luminal bathing solution rather than proton secretion by the mucosa.

Polyamines are naturally occurring compounds found in a variety of tissues, including the stomach (1). Some of the polyamines inhibit acid secretion by the stomach and spermine appears to be the most potent of the compounds tested thus far (2, 3). Spermine has been shown to inhibit acid secretion by isolated bullfrog stomach and the pylorus-ligated rat model (2, 3). Because of its apparent effect on the luminal side of the mucosa, spermine has been suggested as a potential therapeutic agent for inhibition of acid secretion. However, the reported inhibition of acid secretion may be only apparent in that back diffusion of acid has not been excluded and the presence of multiple charged amino groups may in themselves act as anionic receptors causing unanticipated effects. The purpose of this study was to examine the effects of spermine on acid secretion and back diffusion by isolated gastric mucosa in order to more fully understand the actions of this polyamine.

Methods. New Zealand white rabbits (2 to 3 kg), which had been allowed free access to a standard diet and water, were killed by a sharp blow to the neck. A portion of the greater curve of the stomach adjacent to the spleen was excised and mounted on a Lucite half-chamber which allowed isolation of the mucosa, while the luminal side was bathed in oxygenated Ringer's solution (4). The serosa and muscularis propria were sharply stripped away to the level of the muscularis mucosa. The remaining mucosa was clamped between two half-chambers perfused individually with gas-lift circulating systems maintained at 37.5°C. Both sides of the mucosa were bathed in identical Ringer's solution which was bubbled with 100% O_2 and contained the following (in m*M*): Na, 136; K, 5; Ca, 0.5; Mg, 1.2; Cl, 144.4; and glucose, 10.

The short-circuit current and transmural electrical potential difference were measured and electrical resistance calculated as described previously (4). Each tissue served as its own control and after baseline measurements, spermine was added to either the mucosal or serosal sides of the tissue.

The rates of acid secretion and luminal acid loss were measured by the pH stat method. Stimulated acid secretion was achieved by the addition of either histamine or theophylline to the serosal side. Titrations for acid secretion were performed at pH 7 (unless stated otherwise) using NaOH, 0.01 *N*, and for luminal acid loss at pH 4, using HCl, 0.01 *N*, as the titrants (4, 5). Prior to the addition of spermine to the mucosal side, the compound was pretitrated to the appropriate pH in a stirred titrator cup. Preliminary experiments (using a Radiometer PHA943 titragraph module) indicated that the pK of spermine added to Ringer's solution under the present experimental conditions was about 7.4.

The rates of mucosal and serosal release of CO_2 was measured using Al-Awaqati's and associates modification of Maffly and associates method (6) with a Wescan differential

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conductivity meter (model 214-100). During these experiments, the mucosal and serosal sides of the tissues were bathed in phosphate-buffered (pH 7) Ringer's solution.

Statistical analyses were performed using Student's t test for paired variates.

Results. The mean rates of unstimulated acid secretion are shown in Fig. 1. The acid secretory rate did not change significantly in control tissues. Preliminary experiments indicated that incremental additions of spermine, beginning with 0.2 mM, to the mucosal side caused progressive changes in acid secretion with about 1 mM being near maximal. The effects of 5 and 10 mM spermine were similar to that observed for 1 mM. The addition of 1 mM spermine to the mucosal bathing solution was followed by an 80% decrease ($P < 0.001$) in acid secretory rate. However, this effect was transient, lasting no more than 20 min and the mean inhibitory effect lasted 11.6 ± 2.2 min (mean ± SE). Subsequently, apparent acid secretion returned to within 0.4 μeq/hr cm^2 of the basal rate and the mean rate was not significantly different from that observed prior to the addition of spermine. An additional dose of spermine had effects similar to the initial dose. These observations are in contrast to the addition of two separate doses of spermine to the serosal bathing solution, which did not significantly alter the acid secretory rate.

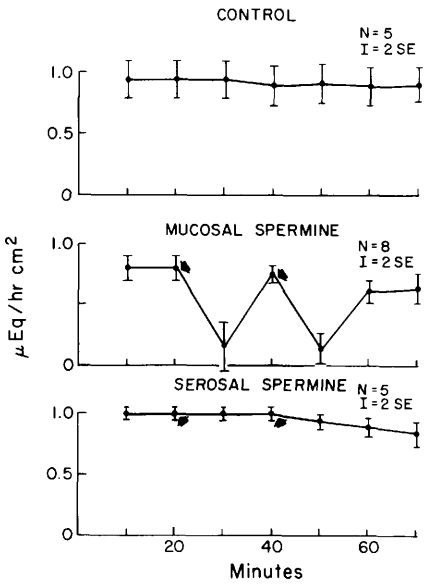

FIG. 1. Effects of spermine on unstimulated acid secretion by fundic mucosa measured at pH 7. Arrows indicate addition of 1 mM spermine. Each point indicates steady-state rate acid secretion over a 10-min interval.

The effects of spermine on the short-circuit current (I_{sc}) and transmural electrical potential difference (PD) are shown in Fig. 2. Addition of the polyamine to the mucosal bathing solution was followed by a persistent decrease in I_{sc} ($P < 0.001$) and a relatively smaller, persistent decrease in PD ($P < 0.01$). These changes persisted despite the apparent recovery of acid secretion and during the subsequent addition of another dose of spermine. Both the I_{sc} and PD were unaffected by the addition of spermine to the serosal side. The tissue electrical resistance (R) before the first addition of spermine to the mucosal side, 95 ± 5 ohm cm^2, increased by 27 ± 3 ohm cm^2 ($P < 0.001$) after the addition of spermine. The second dose of the polyamine did not significantly change the R further. Serosal addition of spermine also did not significantly affect resistance. The I_{sc}, PD, and R of control tissues did not change significantly.

The effects of mucosal spermine also were measured on acid secretion by tissues stimulated with histamine, 45 μM, or theophylline, 5 mM, added to the serosal bathing solution (Fig. 3). Following stimulation by histamine, 1 mM spermine decreased the acid secretory rate by 30% ($P < 0.0025$). Addition of another 1 mM spermine within 5 min of the initial decrease caused a further reduction in acid secretion, reaching a level which was not significantly different from zero. However, within 20 min, apparent acid secretion returned, reaching a level not significantly different from that observed prior to the addition of histamine. Spermine, 1 mM, also caused a decrease in acid secretory rate stimulated by a submaximal concentration of theophylline (7). The initial addition of spermine caused an 83% reduction ($P < 0.001$) in acid secretion. A subsequent addition of 1 mM spermine reduced the acid secretory rate to a level not significantly different from zero; this change was not significantly different from that observed after the addition of the first dose of spermine. Again, within 20 min of adding the second dose of spermine, apparent acid secretion returned to within 0.3 μeq/hr cm^2 of

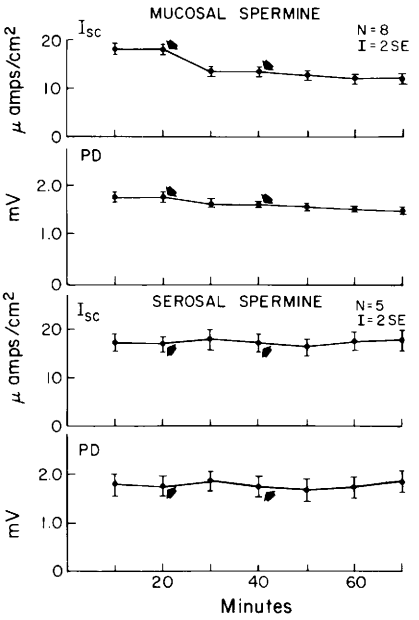

FIG. 2. Effects of spermine on short-circuit ($\mu A/cm^2$) and transmural electrical potential difference (mV) of tissues shown in Fig. 1. Arrows indicate addition of 1 mM spermine.

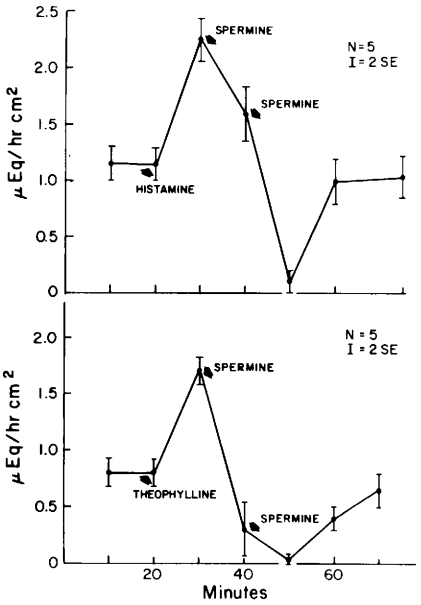

FIG. 3. Effects of spermine on acid secretion measured at pH 7 and stimulated by either histamine (arrow, 45 μM) or theophylline (arrow, 5 mM). Arrows for spermine indicate the addition of 1 mM to the mucosal bathing solution. Each point indicates steady-state rate acid secretion over a 10-min interval.

the pretheophylline rate. As expected from these data, tissues pretreated with spermine, 1 mM, are resistant to the acid stimulatory effects of histamine (N = 3) and theophylline (N = 2).

The effect of a 10-fold increase in K^+ concentration, 50 mM, added to the mucosal side of the tissues treated with spermine is shown in Fig. 4. Potassium was added as soon as the acid secretory rate reached a nadir in response to 1 mM spermine. The rate of acid secretion increased in response to K^+, reaching a level not significantly different from that observed before the addition of spermine. In contrast to tissues not exposed to the high concentration of K^+ (Fig. 1), a subsequent addition of 1 mM spermine had a variable effect. Four tissues showed transient inhibition of acid secretion amounting to no more than a 30% decline and only two of these tissues manifested rebound acid secretion; the remaining tissue was unresponsive to spermine. Prior to the addition of K^+, the I_{sc}, PD, and R changed in the same manner as shown in Fig. 2. However, after the addition of K^+ and return of acid secretion to near basal rates, the I_{sc}, PD, and R also returned to their basal values. When tissues were pretreated with 50 mM K^+, spermine had identical effects on H^+ secretion but did not alter I_{sc}, PD, or R.

Two observations indirectly suggested that the rebound acid secretory effect occurring after the initial inhibition caused by spermine

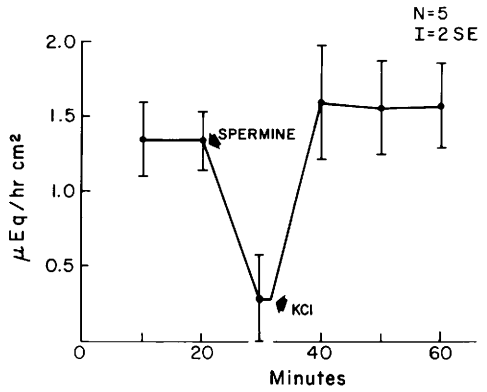

FIG. 4. Effect of KCl (arrow, 50 mM) added to the mucosal side on acid secretion measured at pH 7 of tissues treated with spermine (arrow, 1 mM). Each point indicates steady-state rate H^+ secretion over a 10-min interval except the 30-min value. K^+ was added 2 min after the acid secretion rate reached its nadir.

(in the absence of extra K^+) may not be due to the secretion of H^+ by the tissue. First, the I_{sc} and PD did not return to their basal levels as the rebound acid secretory phenomenon occurred. Second, a subsequent dose of spermine had virtually the same effect on apparent acid secretion as the initial dose (Fig. 1). However, the acid secretory rebound phenomenon casts some doubt on the validity of the observation of K^+ reversing the spermine-induced inhibition of acid secretion. The reversal may have been due to the acid secretory rebound effect and not to the addition of K^+. Indirect evidence, however, suggests that K^+ does have an effect because after its addition the I_{sc}, PD, and R returned to basal levels. Further studies were done in order to help clarify the effect of spermine on acid secretion. These experiments involve the effects of spermine on the rate of CO_2 release in a neutral pH mucosal bathing solution as well as net H^+ flux in an acidic mucosal bathing solution.

Mucosal (J_m) and serosal (J_s) rates of CO_2 release were alternately measured before and after the addition of spermine, 2 mM, to the mucosal bathing solution of five tissues (Fig. 5). The time frame in these experiments were similar to those in Fig. 1. Under the conditions used for these experiments, the difference (J_{net}), between J_m and J_s equals the rate of acid secretion (4). Prior to the addition of spermine, J_{net} was -1.3 ± 0.2 μmole/hr cm^2, but after spermine, J_{net} was nonexistent. This change was due to a decrease in J_s by 1.4 ± 0.2 μmole/hr cm^2 ($P < 0.001$), J_m having decreased by only 0.1 ± 0.1. CO_2 release by control tissues ($N = 5$) measured over the same time intervals showed that J_m changed by 0.2 ± 0.2 and J_s changed by 0.3 ± 0.2 μmole/hr cm^2.

Net luminal loss of acid is known to spontaneously occur when the mucosal bathing solution of isolated fundic mucosa of rabbits is kept at pH 4 (5) (Fig. 6). The rate of luminal acid loss did not change significantly in control tissues. If spermine inhibits the acid secretory rate at luminal pH 4, then a persistent increase in net luminal loss of acid should occur (3). On the other hand, if the acid secretory rebound phenomenon observed at luminal pH 7 also occurs at luminal pH 4, then the rate of net luminal acid loss should be gradually reduced. As shown in Fig. 6, only the former was observed in response to spermine. Furthermore, the spermine-induced increase in net luminal acid loss was reversed by the addition of 50 mM K^+, which corroborates its effects observed at neutral luminal pH. The effect of spermine on net luminal acid loss

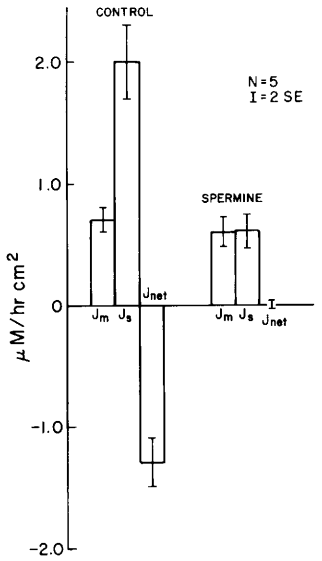

FIG. 5. Effects of spermine, 2 mM, on steady-state mucosal (J_m), serosal (J_s), and net ($J_{net} = J_m - J_s$) CO_2 release by isolated fundic mucosa bathed in pH 7 phosphate-buffered Ringer's solution.

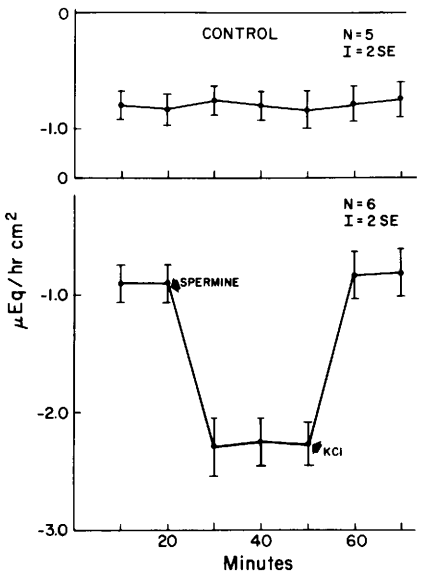

FIG. 6. Effect of spermine, 2 mM (arrow) and KCl, 50 mM (arrow) on luminal acid loss by fundic mucosa measured at pH 4. Each point indicates steady-state rate of acid loss.

was inhibited by pretreatment with K^+, 50 m*M*.

The bidirectional flux of H^+ in fundic mucosa (H^+ secretion and luminal H^+ loss) poses problems in determining the effect of spermine on mucosal permeability to H^+ (or what has been popularly called acid back diffusion). This determination is simplified by using isolated antral mucosa which in the rabbit neither secretes HCO_3^- without this buffer in the serosal bathing solution nor significant amounts of H^+ (8). In the absence of HCO_3^- in the bathing solution, the rate of luminal acid loss by control antral tissues ($N = 5$) did not change significantly, and spermine given as two separate 1 m*M* doses did not have an appreciable effect ($N = 5$). Spermine also did not affect the I_{sc}, PD, or *R*.

Discussion. Spermine inhibits acid secretion by isolated fundic mucosa of the rabbit. This effect occurs when the polyamine is added only to the mucosal side of the tissue. Serosal application is without apparent effect on acid secretion (Fig. 1). These results are in agreement with those previously observed for isolated bullfrog fundic mucosa and the pylorus-ligated rat model (2, 3). However, the present results differ from those reported for stimulated bullfrog mucosa and rat stomach in that the apparent effect of spermine on the spontaneously secreting rabbit stomach was transient, lasting about 10 min, and followed by a rebound with apparent acid secretion returning close to the basal rate. Stimulated tissues also exhibited the inhibitory and rebound phenomenon, but the rebound rate was always much lower than the stimulated secretory rate (Fig. 3).

It is possible that the transient effect of spermine on acid secretion was due to rapid metabolism of the polyamine by the tissue, thus implying a true transient acid inhibitory effect. However, isolated preparations of liver, spleen, kidney, and lung fail to degrade exogenous spermine for up to 5 hr *in vitro* (1). Furthermore, a 5- to 10-fold increase in polyamine concentration also caused transient inhibition of acid secretion, making it unlikely that metabolism of the compound is a major factor. The observation that K^+ reverses the effect of spermine at luminal pH 7 (Fig. 4) and in particular at luminal pH 4 (Fig. 6) also argues against appreciable tissue metabolism of the polyamine.

The effect of spermine on CO_2 release by fundic mucosa suggests that in spite of the apparent rebound in acid secretion, the polyamine causes prolonged inhibition of acid secretion. The production and translocation of H^+ generates an equivalent alkaline tide, readily detectable in the form of HCO_3^- which preferentially diffuses through the serosal side of the tissue (4). Carbon dioxide, being freely permeable, should have equal rates of appearance on the mucosal and serosal side if the CO_2 is the result of tissue metabolism alone. If the serosal bathing solution is buffered, diffusing HCO_3^- (which is produced on a 1:1 basis for every proton secreted) is converted to CO_2, giving rise to a greater rate of appearance of CO_2 on the serosal compared to the mucosal side of the tissue. The difference in rates of CO_2 appearance on the mucosal and serosal sides has been shown to equal the rate of net acid secretion (4). Spermine does not significantly affect the mucosal rate of CO_2 appearance but decreases the rate of serosal CO_2 appearance to a level that does not significantly differ from the mucosal rate (Fig. 5). This change reflects inhibition of HCO_3^- diffusion and suggests that the release of CO_2 from the serosal side after exposure of spermine is the result of tissue metabolism only.

It has been suggested that gastric H^+, K^+-ATPase provides the necessary energy for the separation of H^+ from OH^- and carbonic anhydrase is involved in the subsequent conversion of OH^- to HCO_3^- (9). Generation of the alkaline tide could be prevented if there is no physical separation of H^+ and OH^-. This situation presumably occurs in truly resting mucosa and in stimulated mucosa where the transport of H^+ from the cell interior into the lumen is prevented or possibly in the circumstance of luminal H^+ rapidly diffusing back into the mucosa. Since spermine does not cause detectable diffusion of H^+ from the lumen, it appears that spermine either interferes with H^+, K^+-ATPase activity or the transport of H^+ into the lumen. The latter conclusion is supported by a previous report indicating that rather than affecting gastric H^+, K^+-ATPase activity in microsomal vesicles, spermine alters the intravesicular transport of H^+ (2).

Another observation also suggests spermine causes a persistent inhibition of acid secretion. Isolated gastric mucosa of the rabbit exhibits

a higher spontaneous passive permeability than gastric mucosa of other species (10). Thus, when the luminal pH is decreased from 7 to 4, luminal acid loss (so-called "back diffusion") occurs. Under the experimental conditions determined for fundic mucosa, the flux is a net one, representing the algebraic sum of acid secretion into the lumen and acid diffusion from the lumen into the tissue. At pH 4, spermine causes an increase in net luminal acid loss (Fig. 6). This observation could be due either to inhibition of acid secretion or an increase in tissue permeability, permitting greater acid diffusion. Agents which alter permeability of antral mucosa also do so in a qualitatively similar manner for fundic mucosa (10). The fact that the tissue electrical resistance does not decrease upon exposure of antral mucosa to spermine and the fact that spermine does not affect acid diffusion by antral mucosa suggests that the polyamine inhibits acid secretion by fundic mucosa. If the acid inhibitory effect of spermine was transient, one would expect luminal acid loss by fundic mucosa to transiently increase and decrease. However, spermine caused a persistent increase in luminal acid loss.

The inhibition of acid secretion caused by the polyamine was consistently reversed by the addition of K^+ and this reversal was observed at both pH 7 and 4. In addition to the reversal of acid secretion, K^+ also caused the short-circuit current and transmural electrical potential difference to return toward basal levels.The effect of K^+ supports the concept that spermine inhibits secretion by interfering with a K^+ site located at the secretory membrane.

The apparent acid secretory rebound phenomenon observed after the addition of spermine is most likely related to the formation of H^+ in the luminal bathing solution. Spermine acts as a buffer with one of its apparent dissociation constants (pK) approximating 7.4 under the present experimental conditions. However, the spermine was pretitrated to the luminal pH of the experimental conditions before addition of the polyamine. The most likely explanation for the acid-producing effect of spermine is in part found in Kimura and Sakonaka's data (11). They recently reported that spermine readily binds with HCO_3^-, releasing H^+, and gastric mucosa is known to secrete HCO_3^- in addition to acid. Such an effect would not necessarily be detectable by conductometric CO_2 measurements, especially if some HCO_3^- continues to appear to the serosal side of the tissue.

The present data point to a hazard in the interpretation of apparent effects of an agent on acid secretion by the gastric mucosa. Apparent inhibition or stimulation of acid secretion may be deceptive in terms of the action(s) of a given agent. Spermine has an appealing effect on acid secretion because of its local effectiveness on the luminal side. The potential therapeutic effect of specifically spermine may be mitigated by its ability to acidify the gastric lumen at neutral pH even though it inhibits acid secretion. However, as suggested by prior (2, 3) and present data, the apparent proton-forming capacity of spermine is negligible when there is some acid in the lumen.

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