PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 172, 308–315 (1983)

Inhibition of Guinea Pig Gastric Microsomal Potassium-Stimulated ATPase by Nolinium Bromide (41562)

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Abstract. Although nolinium bromide (NB) [2-(3,4-dichlorophenylamino)-quinolizinium bromide] inhibited histamine-stimulated acid secretion *in vivo* in the pylorus-ligated guinea pig, no inhibition of basal or histamine-stimulated acid secretion from the isolated guinea pig gastric fundic disk was observed at serosal or mucosal concentrations from 0.1 to 200 μM. NB did not inhibit carbonic anhydrase in whole stomach homogenates of guinea pig, but did inhibit the potassium-stimulated gastric microsomal ATPase. The IC_{50} value (compound concentration giving 50% inhibition of the rate of ATP hydrolysis) for the net stimulated reaction (activity with KCl minus activity without KCl) was 65 μM (95% confidence limits = 42–101 μM). The basal reaction (no added KCl) was not inhibited. Inhibition of ATPase by NB was not due to its bromide ion, since NaBr did not, while the chloride analog of NB did, inhibit the enzyme. It was concluded that NB has no direct histamine-H_2 receptor blocking activity. Whether ATPase inhibition contributes to its gastric acid antisecretory property is uncertain because of the lack of inhibitory activity in the isolated gastric disk assay.

Nolinium bromide[1] (NB)[2] [2-(3,4-dichlorophenylamino)quinolizinium bromide] inhibited basal gastric acid secretion in the pylorus-ligated rat at oral doses from 25 to 150 mg/kg (1) and histamine- and pentagastrin-stimulated secretion (ED_{50} values of 84.9 and 77.5 mg/kg po, respectively) (2). Acid secretion stimulated by carbachol or insulin was not as sensitive (ED_{50} values of >750 and 390 mg/kg po, respectively), indicating the non-anticholinergic nature of this agent. In the rat NB inhibited gastric ulcer formation due to pylorus-ligation, aspirin, and stress (1). In man NB inhibited pentagastrin-induced gastric acid secretion (3). NB has been shown to have antispasmodic action in the gastrointestinal tract of rabbit, rat, and dog (4). NB was also found to possess antiinflammatory activity in the carrageenin-induced hindpaw edema model in the rat at doses of 150 and 300 mg/kg po (5).

The antisecretory action of NB has been attributed to histamine-receptor blockade in gastric tissue (6), based on the finding that the compound inhibited only histamine-stimulated adenylate cyclase and not the activity stimulated by prostaglandin PGE_2, sodium fluoride, or 5'-guanylimidodiphosphate. Although this specificity was analogous to that observed with the histamine-H_2 receptor antagonist, cimetidine, inhibition by NB was noncompetitive and the possibility existed that a site other than the histamine-binding side was involved in the compound's action. We have further investigated the basis of the antisecretory action of NB in studies using the isolated guinea pig gastric fundic disk and two enzymes from guinea pig stomach, carbonic anhydrase and $(H^+ + K^+)$-ATPase. Our results suggest that the agent does not interact at the histamine receptor. Although NB did inhibit the proton-transport ATPase, its lack of activity in the gastric disk assay precludes ascribing its *in vivo* antisecretory action directly to effects on enzymes of gastric secretion. A preliminary report of these results has appeared (7).

Materials and Methods. *Chemicals.* EU-3369, cimetidine and NB were prepared at Norwich Eaton Pharmaceuticals. These compounds were dissolved in distilled water (*in vitro* studies) or 0.5% (w/v) methylcellulose (*in vivo* studies) on the day of use. Methylcellulose (Methocel 4000 cps) was purchased from Dow Chemical (Williamsville Center, N.Y.) and histamine diphosphate from City Chemical Corp. (New York, N.Y.).

Animals. Male albino Hartley guinea pigs (Crt:COBS(HA), Elm Hill Breeding Labs, Chelmsford, Mass.) were maintained on

[1] U.S. Patent 3,763,174 (1973).

[2] Abbreviation used: NB, nolinium bromide.

0037-9727/83 $1.50

Wayne Rabbit Pellets (Wayne Pet Foods, North Platte, Neb.) and water containing 0.2% (w/v) ascorbic acid *ad libitum*. For isolated gastric disk studies and the pylorus-ligated model the animals weighed between 225 and 500 g and for the enzyme work weights ranged from 300 to 700 g. All animals were fasted overnight except those used to prepare gastric ATPase.

Pylorus-ligated guinea pig. The gastric acid antisecretory effect of NB was determined in the guinea pig according to the method of Shay *et al.* (8). To study effects on basal secretion, fasted guinea pigs were dosed orally by metal tube with 10, 30, 100, and 300 mg/kg of NB. One hour later each animal was anesthetized with ether. The pylorus was ligated with 3-O surgical silk and the incision closed with wound clips. Four hours later (5 hr after dosing) animals were killed by CO_2 inhalation. Stomach contents were carefully collected and clarified by centrifugation (clinical centrifuge, 3/4 power, 10 min). Volume and titratable acidity were determined. The effect of NB on histamine-stimulated gastric acid secretion was determined in a similar fashion. After pylorus-ligation each animal received a subcutaneous injection of 80 μg/kg histamine in a volume of 1 ml/kg. Three additional injections were administered at 45-min intervals. Three hours after ligation (4 hr after dosing) the animals were killed as above.

Isolated guinea pig gastric disk. A modification of the methods of Sjöstrand *et al.* (9) and Holton and Spencer (10) was used. In brief, the fundic portion of a guinea pig stomach was stripped of the muscle layers and mounted, mucosa-in, over the open end of a glass tube. This was placed at 37°C in a 25-ml Anderson tissue bath filled with modified Krebs solution (serosal solution = 118 m*M* NaCl, 4.69 m*M* KCl, 2.52 m*M* $CaCl_2$, 25 m*M* $NaHCO_3$, 1.18 m*M* KH_2PO_4, 1.16 m*M* $MgSO_4$, 25 m*M* D-glucose, pH 7.4 at 22°C). Inside the tube was placed 5 ml of mucosal solution (143 m*M* NaCl, 5.87 m*M* KCl, 2.52 m*M* $CaCl_2$, 1.16 m*M* $MgSO_4$, 10 m*M* D-glucose, pH 5.8 at 22°C). The serosal solution was gassed at approximately 20 ml/min with 95% O_2–5% CO_2 and the mucosal solution with 100% O_2 at approximately 17 ml/min via a glass tube immersed to just above the disk surface. Compounds were added to either the serosal or mucosal solution and maintained throughout the experiment. In a typical experiment the disk was allowed to secrete acid for a 30-min equilibration period, then for another 90 min to establish the basal secretion rate. For work with stimulated tissues, histamine was added to the serosal solution at this point and removed after 30 min by flushing with at least 100 ml of serosal solution. In all experiments the mucosal solution was removed every 30 min and its pH determined. Since acid concentrations calculated from pH readings were not significantly different from those determined by titration with standard base, it was concluded that the simpler former method was valid under these experimental conditions. Acid secretion rates are expressed as microequivalents per square centimeter of tissue surface per hour.

Carbonic anhydrase preparation and assay. A procedure modified from that of O'Brien *et al.* (11) was used to prepare a fraction of guinea pig stomach with carbonic anhydrase activity. A single stomach was rinsed in tap water and stirred in several changes of 2 *M* NaCl for 1–2 hr to remove blood. A 10–26% homogenate in cold distilled water was made (Polytron Type PT 10-20-3500, Brinkmann Instruments, Westbury, N.Y.) and centrifuged 10 min at 12,000*g* (Sorvall SS34 rotor, 4°C). In some preparations partial removal of mitochondria was done by an additional 17,000*g* centrifugation. The supernatants of these centrifugations were kept cold and used on the day of preparation as the source of carbonic anhydrase. Protein concentration was determined by the method of Lowry *et al.* (12) using bovine serum albumin as standard. For assay, the method of Livesley (13) was used.

$(H^+ + K^+)$-ATPase preparation and assay. Parietal cells were obtained from guinea pig stomachs by the procedure of Forte *et al.* (14) and 14 or 19 volumes (ml/g) of buffer [200 m*M* sucrose, 0.2 m*M* ethylenediamine tetraacetic acid, and 0.2 m*M* 1,4-piperazine diethanesulfonic acid (PIPES), pH 6.8 at 22°C] were added. The method used to purify the microsomes containing the ATPase was similar to that of Sen and Ray (15). Cells were broken by ten strokes of a Dounce homogenizer. The homogenate was centrifuged

(8000*g*, 10 min) and the supernatant was saved. The pellet was resuspended in half the original volume, rehomogenized, and recentrifuged twice more. Combined supernatants were layered over 37% (w/v) sucrose–0.2 m*M* PIPES (pH 6.8 at 22°C) and centrifuged at least 5 hr at 55,000–78,000*g*. The microsomal band at the buffer–sucrose interface was diluted 1:2 with buffer and centrifuged 90 min at 78,000*g*. Pellets were resuspended in buffer or 0.2 m*M* PIPES (pH 6.8 at 22°C) and stored at −20°C. Protein was determined as above. The presence of a prominent 100,000-molecular weight band equal to 16% of the total protein was shown by polyacrylamide gel electrophoresis in 1% (w/v) sodium dodecylsulfate as described by Fairbanks *et al.* (16) and Ganser and Forte (17). The lack of significant mitochondrial contamination was shown by assaying for the mitochondrial marker enzyme, succinic dehydrogenase, according to Green *et al.* (18).

ATPase was assayed by the method of Lee *et al.* (19) in 1-ml reaction mixtures containing 25 m*M* sucrose, 20 m*M* Tris/HCl (pH 7.4 at 22°C), 5 m*M* $MgCl_2$, and 2 m*M* Na_2ATP. Stimulated reactions contained 20 m*M* KCl. Reactions were started by the addition of microsomes (88 or 109 μg/ml final concentration), run for 30 min at 37°C, and stopped by addition of 0.1 ml of cold 50% trichloroacetic acid followed by 3.9 ml 0.125 *M* sodium acetate buffer (pH 4.0 at 22°C). Precipitated protein was removed by low-speed centrifugation or filtration through 0.45 μm ultrafilters. Inorganic phosphate was determined by the Lowry and Lopez method (20).

Calculations. Effects of NB on the volume of gastric secretion, acid concentration, and total acid in the pylorus-ligated guinea pig model were assessed by analysis of variance and Dunnett's test. Other results were analyzed by *t* tests. A probability level of less than 5% was considered significant.

Results. *Antisecretory activity in the guinea pig.* Since *in vitro* mechanism studies were done with guinea pig materials, it was important to demonstrate the gastric acid antisecretory effect of NB in this species. Single oral doses of NB (30–100 mg/kg) did not significantly inhibit basal gastric secretion volume, acid concentration, or total acid in the pylorus-ligated guinea pig model (data not

TABLE I. EFFECT OF ORAL NOLINIUM BROMIDE ON TOTAL AND NET HISTAMINE-STIMULATED GASTRIC SECRETION IN THE PYLORUS-LIGATED GUINEA PIG

Oral dose of nolinium bromide (mg/kg)	Dose of histamine (μg/kg sc)	Number of animals	Volume (ml/100 g BW[a])			Acid concentration (meq/ml)			Total acid (meq/3 hr/100 g BW[a])		
				Percentage of control			Percentage of control			Percentage of control	
			Mean ± SE	Total	Net	Mean ± SE	Total	Net	Mean ± SE	Total	Net
0 (Methocel)	0 (Saline)	5	1.83 ± 0.370	45[b]	NA[c]	0.075 ± 0.011	57[b]	NA[c]	0.146 ± 0.045	27[b]	NA[c]
0 (Methocel)	80 × 4 (Control)	5	4.03 ± 0.459	100	100	0.132 ± 0.004	100	100	0.532 ± 0.056	100	100
50	80 × 4	4	4.50 ± 1.04	112	121	0.097 ± 0.014[d]	73	51[b]	0.370 ± 0.025	70[b]	58[b]
125	80 × 4	6	3.29 ± 0.185	82	66	0.076 ± 0.004	58[b]	7[b]	0.251 ± 0.020	47[b]	27[b]
300	80 × 4	3	3.38 ± 0.120	84	71	0.088 ± 0.004	67[b]	23[b]	0.298 ± 0.021	56[b]	39[b]

Note. Stomach contents were collected 3 hr after pylorus-ligation (45 min after the final histamine dose). Net secretion was obtained by subtracting basal secretion values (from the group dosed only with saline) for volume, concentration, and total acid from the corresponding individual values of the histamine-dosed groups. Net values less than zero were taken as zero. The resulting net histamine-induced secretion values are expressed as a percentage of the control (no nolinium bromide) group.

[a] BW = body weight.
[b] Significantly ($P < 0.05$) different from control (histamine but no nolinium bromide) value by Dunnett's test and one-way analysis of variance.
[c] Not applicable.
[d] 5 animals.

TABLE II. EFFECT OF NOLINIUM BROMIDE ON ACID SECRETION FROM THE HISTAMINE-STIMULATED GUINEA PIG GASTRIC FUNDIC DISK

Nolinium bromide concentration (μM)	Number of tissues	Acid secretion rate (μeq/hr/cm^2)					
		Prehistamine		Gross posthistamine		Net posthistamine	
		Mean ± SE	Percentage of control	Mean ± SE	Percentage of control	Mean ± SE	Percentage of control
Serosal nolinium bromide							
0 (control)	11	3.49 ± 0.350	100	6.56 ± 0.452	100	3.20 ± 0.536	100
1	6	3.25 ± 0.208	93	7.23 ± 0.761	110	3.98 ± 0.805	124
10	4	3.50 ± 0.675	100	7.62 ± 1.36	116	4.12 ± 0.863	129
50	4	3.22 ± 0.310	92	6.97 ± 0.573	106	3.75 ± 0.758	117
100	4	2.36 ± 0.578	68	6.32 ± 0.276	96	3.95 ± 0.651	123
200	3	10.67 ± 0.898	308[a]	13.62 ± 1.21	208[a]	2.95 ± 1.60	92
Mucosal nolinium bromide							
0 (control)	21	2.72 ± 0.204	100	6.25 ± 0.512	100	3.53 ± 0.500	100
1	6	3.13 ± 0.649	115	6.29 ± 1.20	101	3.16 ± 0.609	90
10	6	2.10 ± 0.451	77	5.27 ± 1.05	84	3.17 ± 0.983	90
50	4	1.98 ± 0.182	73	6.19 ± 0.716	99	4.21 ± 0.804	119
100	3	4.71 ± 0.079	173[a]	11.7 ± 0.470	187[a]	6.97 ± 0.392	198[a]
200	3	4.40 ± 1.56	161	9.44 ± 0.220	151	5.04 ± 1.35	143

Note. Tissues were stimulated with 100 μM histamine diphosphate added serosally for 15 min. The indicated concentration of NB was present throughout the experiment.

[a] Significant ($P < 0.05$) increase due to nolinium bromide based on Dunnett's test and one-way analysis of variance.

shown). When gastric secretion was stimulated by the subcutaneous administration of four injections of histamine diphosphate (80 μg/kg), NB had no significant effect on volume, but did significantly reduce by one third to one half the acid concentration and total acid at 125 and 300 mg/kg po (Table I). There was a significant 30% reduction in total acid at 50 mg/kg po as well. The doses causing 50% inhibition of total and net histamine-stimulated total acid were 230 and 48 mg/kg, respectively.

Guinea pig gastric disk. The isolated guinea pig gastric disk was validated as a system for studying inhibition of gastric acid secretion by results with cimetidine, a known histamine H_2-receptor blocker. Cimetidine, in serosal concentrations of 10, 50, and 100 μM, significantly reduced net histamine-induced acid secretion to 53, 56, and 37% of the control level in this model. Basal secretion was unaffected and mucosal application of cimetidine had no effect (data not shown). NB, in serosal concentrations from 1 to 100 μM, did not affect acid secretion, either basal or histamine-induced (Table II). At 200 μM serosal NB, basal acid was significantly increased threefold. Mucosal concentrations of NB from 1 to 50 μM were without effect, but higher levels (100 and 200 μM) increased both basal and histamine-induced acid secretion rates. Only the increase of up to twofold at 100 μM was significant, however.

Because NB was known to inhibit an enzyme that uses a nucleotide substrate, adenylate cyclase (6), its action as a cAMP phosphodiesterase inhibitor was considered as a possible explanation for the acid stimulation effects seen at 100 and 200 μM. Consistent with this possibility was the finding that a known phosphodiesterase inhibitor, theophylline, also stimulated basal, but not hista-

TABLE III. EFFECT OF NOLINIUM BROMIDE ON GUINEA PIG GASTRIC MICROSOMAL ($H^+ + K^+$)-ATPase

Compound	Concentration (μM)	Percentage inhibition (mean ± SE)[a]	
		Basal reaction	Net K^+-stimulated reaction
Nolinium bromide	25	14 ± 2.7[b]	9.3 ± 7.1[b]
	50	17 ± 3.6[b]	31 ± 2.8[b]
	100	7.9 ± 0.8[b]	75 ± 0.6[b]
	200	14 ± 2.2[b]	86 ± 4.9[b]
	1000	−2.9 ± 4.6	99 ± 1.3[b]
EU-3369	100	4.4 ± 5.4	29 ± 2.2[b]
	500	18 ± 4.8	66 ± 3.1[b]
	1000	0 ± 0	100 ± 0[b]
Cimetidine	200	0.1 ± 2.2	−5.0 ± 10
Atropine sulfate	1000	10 ± 1.7[b]	−19 ± 2.0[b]
Ouabain	2000	10 ± 9.3	−3.4 ± 7.4
N-ethylmaleimide	2000	25 ± 1.3[b]	52 ± 4.2[b]
Oligomycin	200	−6.4 ± 1.0[b]	−19 ± 129
NaBr	100	3.0 ± 5.3	−39 ± 2.4[b]
	500	−11 ± 7.0	9.0 ± 0.9[b]
	1000	−3.4 ± 2.7	3.4 ± 2.1

Note. ATPase activity was determined in triplicate. Control reactions (0 mM KCl) had basal specific activities of 12–14 μmol P_i released/hr/mg and potassium stimulation (20 mM KCl) ranged from 1.2- to 2.6-fold.

[a] Negative values indicate stimulation.

[b] Significant effect ($P < 0.05$) by two-tailed t test (4 df).

mine-induced, acid secretion in the gastric disk system when added to the serosal solution (data not shown).

Effect on carbonic anhydrase. The gastric carbonic anhydrase from unstimulated guinea pigs was not significantly affected by NB at concentrations up to 200 μM (data not shown). Cimetidine was also without effect at 200 μM, but a known inhibitor of this enzyme, *p*-aminomethylbenzylsufonamide (21), reduced activity to less than 10% of control at 200 μM.

Inhibition of gastric microsomal ($H^+ + K^+$)-ATPase. The ATPase found in the microsomal fraction from the stomach of the guinea pig, a source not previously described in the literature, was judged acceptable for studying the effect of NB based on several criteria. As expected of a suitable ATPase preparation, the rate of ATP hydrolysis was linearly dependent on enzyme concentration (between 14 and 114 μg/ml) and time (up to 30 min). The preparation showed a prominent 100,000-molecular weight band on polyacrylamide gel electrophoresis. This band has been identified by Saccomani *et al.* (22) as the ATPase in a similar preparation from hog gastric microsomes. Lack of contamination by mitochondria was indicated by the absence of any enrichment for succinic dehydrogenase activity, a mitochondrial marker enzyme. Oligomycin, a known inhibitor of mitochondrial ATPase, did not inhibit at 0.2 mM (Table III). Ouabain (2 mM), a known inhibitor of the ($Na^+ + K^+$)-ATPase, was also without effect (Table III). On the other hand, *N*-ethylmaleimide, a sulfhydryl-directed inhibitor of hog gastric microsomal ATPase, did inhibit the guinea pig enzyme (52% at 2 mM). By all these properties the guinea pig enzyme was comparable to the microsomal ($H^+ + K^+$)-ATPase identified in other species (17, 19, 22–26). The amount of stimulation by potassium (1.2- to 2.6-fold) observed with the guinea pig enzyme was also similar to that observed with the ATPases from other species.

NB, at concentrations from 0.025 to 1 mM, produced a concentration-dependent inhibi-

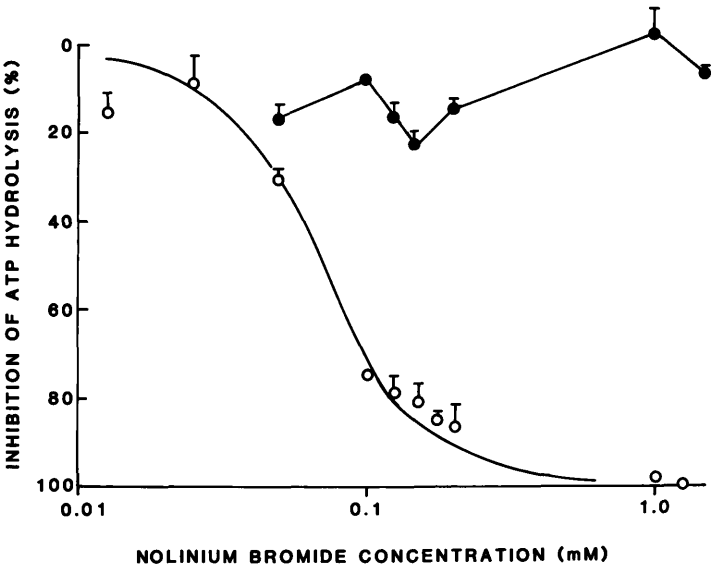

FIG. 1. Inhibition of guinea pig gastric microsomal ATPase by nolinium bromide. The net stimulated reaction (empty symbols) was determined by subtracting the hydrolysis seen without added 20 m*M* KCl (basal reaction, filled symbols). Basal enzyme specific activity ranged from 12 to 14 μmole/hr/mg and KCl increased the rate 2.1- to 2.4-fold. Data are given as the mean ± SE (bars) for three determinations.

tion of the net potassium-stimulated ATPase reaction (Table III). At 1 m*M* the stimulated reaction was completely inhibited. Over the same concentration range, the basal reaction was inhibited less than 20% (Fig. 1). The IC_{50} value (concentration giving 50% inhibition) for NB's effect on the net stimulated reaction was 65 μ*M* (95% confidence limits = 42–101 μ*M*). The activity of NB was not due to its bromide component since NaBr was inactive at 1 m*M* and the chloride analog (EU-3369) of NB also inhibited the net stimulated reaction. Two known gastric antisecretory agents, cimetidine and atropine, were not inhibitors of this gastric ATPase (Table III).

Discussion. NB has been previously reported to inhibit basal and stimulated gastric acid secretion in the rat (1, 2). Results reported here show that it is also active in the guinea pig, at least for secretion stimulated by histamine. The ED_{50} value of 230 mg/kg po in the guinea pig was somewhat higher than that found in the rat, 85 mg/kg po (2). In both species greater effects on acid concentration than on secretion volume were observed.

Although NB decreases acid secretion stimulated by histamine in two species, *in vitro* results presented here indicate that the compound is not a direct histamine-H_2 receptor antagonist like cimetidine. The gastric fundic disk responds to histamine by markedly increasing acid production and this response is inhibited by cimetidine applied serosally. NB, however, did not inhibit and, indeed, stimulated acid production at its highest concentrations.

We (6) reported that the histamine-stimulated adenylate cyclase of guinea pig gastric homogenate was noncompetitively inhibited by NB with an IC_{50} value of approximately 100 μ*M*. It was noted that neither the basal reaction nor activity stimulated by prostaglandin PGE_2, sodium fluoride, or 5'-guanyliminodiphosphate was affected by the compound and that this inhibition pattern was similar to that observed with cimetidine, suggesting a similar site of action. That this similarity was due to interaction at the same histamine-H_2 receptor does not seem tenable in light of the present results.

A potassium-stimulated Mg^{2+}-dependent ATPase has been identified in gastric microsomes of several species, including frog (17, 24), rabbit (25, 26), hog (22), dog and cat (19), and man (27). This report extends the list to include the guinea pig. The enzyme is located

on the lumenal surface of the acid-secreting parietal cell (28), catalyzes the exchange of hydrogen and potassium ions, and is likely to be the terminal effector pump in the acid secretion process (29, 30). NB inhibited the potassium-stimulated reaction catalyzed by the ATPase with moderate potency, but did not affect basal activity. Fellenius *et al.* (27) have recently reported that a benzimidazole derivative, H 149/94, was able to inhibit the K^+-ATPase from pig mucosa with an IC_{50} value of approximately 90 μM. This is comparable to the 65 μM value found for NB. A related compound, H 83/69, has also been reported to inhibit the ATPase (31, 32). Like NB both compounds inhibit gastric acid secretion *in vivo*.

The possibility that NB exerts at least part of its gastric acid antisecretory effect by inhibition of the proton ATPase is apparently not consistent with the lack of inhibitory activity in the isolated gastric disk. Lack of inhibition is not due to inadequate concentrations (200 μM maximum), since blood levels do not exceed 10 μM on chronic oral administration of effective doses (unpublished data). Furthermore the 200 μM concentration used in the disk experiments is near the solubility limit of the compound. The lack of inhibition may be related to the fact that NB is an ionic compound which would have limited access to relevant enzymes (adenylate cyclase and ATPase) in whole tissue experiments. It is possible that the active form of this compound *in vivo* is a metabolism product. Metabolism of NB is extensive and complex, resulting in over 10 compounds. The chief urinary metabolite accounts for only 5% of the administered NB (33). No data is presently available on the effect of NB metabolites on the gastric ATPase. An alternative explanation for the discrepancy between the gastric disk and ATPase results is also recognized, namely that NB's antisecretory action *in vivo* is not due to ATPase inhibition.

Since NB has been shown to inhibit two gastric enzymes, an ATPase and adenylate cyclase, and may also inhibit cAMP phosphodiesterase at high concentrations, it is relevant to ask if there is a common denominator. A possible key may lie in the use, by all three enzymes, of adenosine-containing substrates. NB could, in its quinolizinium ring and the substituted amino group at position 2, mimic the purine ring sufficiently to bind to adenosine sites on all three enzymes. It is of interest that other quinoline and isoquinoline compounds have been shown to inhibit ($Na^+ + K^+$)-ATPase (34, 35). The possibility exists that NB may inhibit other ATPases besides the gastric enzyme and preliminary work supports this notion (36). The antispasmodic action of this compound could be related to inhibition of an ATPase involved in the contraction of smooth muscle.

The authors thank Richard B. Moore for able execution of experiments with the guinea pig fundic disk, Douglas A. Smart and Teresa A. Generous for the ATPase work, and Sandra M. Jones, Kenneth R. Bonk, and Richard H. Burns for the *in vivo* studies.

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Received May 17, 1982. P.S.E.B.M. 1983, Vol. 172.