Action of Nicotine on H^+ Secretion, Potential, and Resistance in Isolated Bullfrog Gastric Mucosa[1] (34659)

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The study of the mechanism of gastric acid secretion in the whole animal is handicapped by an inability to isolate the fundic mucosa from the influence of variable control mechanisms such as blood flow, nervous factors, and humoral substances. While the effects of nicotine on the autonomic nervous system have been extensively studied since its first exact analysis by Langley and Dickinson (1), the actions of this agent on the gastric mucosa have received little attention. The purpose of the present investigation was to determine the effects of nicotine on H^+ secretion, transmucosal potential difference, and resistance in the isolated bullfrog gastric mucosa.

Methods and Materials. The bullfrog, *Rana catesbeiana,* was pithed, the stomach was removed, and opened along the lesser curvature. Fundic gastric mucosa was separated from the muscular layers of the stomach and the intact mucosal membrane was mounted between two Lucite chambers as described previously (2). The total exposed surface area of the fundic mucosa was 2.0 cm^2. The bathing solutions were immediately placed into the chambers and continuously bubbled with 95% O_2 and 5% CO_2. The solutions were of the following composition (mmoles/liter): nutrient (serosal)—Na^+, 90.5; K^+, 5.0; Ca^{2+}, 2.0; Mg^{2+}, 1.5; Cl^-, 82.0; $H_2PO_4^-$, 0.5; HPO_4^{2-}, 1.0; HCO_3^-, 20.0; and glucose, 5.0; secretory (mucosal)—Na^+, 85.0; K^+, 5.0; Cl^-, 90.0; and sucrose 13.0. The pH and osmolarity of the oxygenated nutrient solution were 7.4 and 196 mOsm/liter, respectively. All incubations were carried out at room temperature (23–25°). The rate of acid secretion was determined by a recording pH-stat autoburette titrator (Radiometer, Copenhagen) and expressed in microequivalents of hydrogen ion secreted per square centimeter of gastric mucosa per hour (μeq of $H^+/cm^2 \cdot hr$). The base titrant was approximately 0.0052 *N* NaOH and the normality was determined by titration with a standard HCl solution at regular intervals. The transmucosal electrical potential difference (PD) was measured by a pair of matched calomel electrodes, with renewable KCl junctions, connected to an Esterline Angus Speed Servo recording potentiometer. The secretory side was always negative with respect to the nutrient side. Resistance was calculated from the change in the transmucosal PD obtained by sending 40 μA of current in either direction through a pair of agar–electrolyte electrodes of the following composition: Pb/saturated PbAc/0.1 *M* NaCl/0.5 *M* Na_2SO_4/0.1 *M* NaCl in 2% agar. Nicotine,[2] adjusted to pH 7.4, was added to the nutrient solutions and the dose was expressed as the final molar concentration of the base.

Results. Control observations. All fundic gastric mucosae, incubated under the conditions described, exhibited basal or spontaneous acid secretion. Control experiments were conducted for 140 min without addition of nicotine in six mucosae. The average H^+ secretory rate (μeq/$cm^2 \cdot hr$) during the last 4-min period [3.28 $\pm$ 0.15 ($\pm$ SE)] was not significantly different from that during the first 4-min period (3.34 $\pm$ 0.16) ($p > 0.1$).

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[2] Nicotine base obtained from Matheson Coleman & Bell, Division of Matheson Co., Inc., Norwood, Ohio.

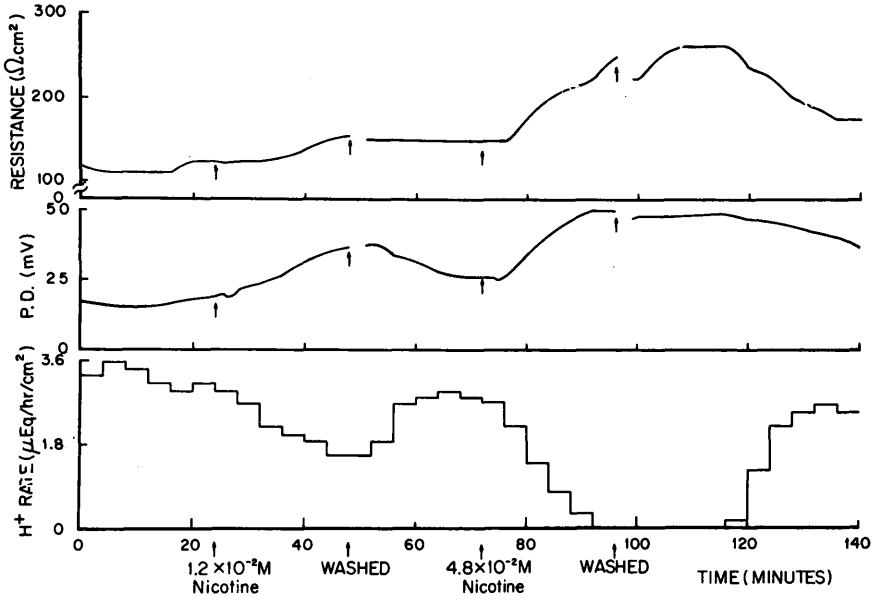


FIG. 1. Effect of 1.2 × 10^{-2} *M* and 4.8 × 10^{-2} *M* nicotine on basal H^+ secretion, transmucosal PD, and resistance in isolated bullfrog gastric mucosa.

The mucosal side was electrically negative with respect to the serosal side by 12–22 mV immediately after mounting the mucosa. The transmucosal PD and resistance showed no significant difference in the course of the experiments, but there were relatively low PD values during the first 10 min and a gradual increase in resistance with time observed.

Nicotine. Figure 1 illustrates the effects of 1.2 × 10^{-2} *M* and 4.8 × 10^{-2} *M* nicotine on H^+ secretory rate, transmucosal PD, and resistance. As shown, 1.2 × 10^{-2} *M* nicotine produced about 50% inhibition of H^+ secretory rate with an increase in transmucosal PD and resistance. Following the removal of nicotine, H^+ secretory rate increased with a fall in transmucosal PD. Subsequent addition of 4.8 × 10^{-2} *M* nicotine to the nutrient resulted in a reduction of H^+ secretory rate to zero and an increase in transmucosal PD and resistance. Removal of the nicotine resulted in a return of H^+ secretory rate to almost the previous level with a fall in transmucosal PD and resistance.

Since the H^+ secretory rate which could be attained following removal of the first dose of nicotine was often lower than the pretreatment level, and the transmucosal PD and resistance were higher, only one dose of nicotine was tested in each experiment to avoid the possible modification of the response to the second dose in the rest of the experiments reported. The comparative effects of four doses of nicotine are summarized in Table I. All doses of nicotine used produced significant inhibition of basal H^+ secretion which could be reversed following removal of nicotine from the bathing medium. The inhibition of H^+ secretion was dose-related and was accompanied by an increase in transmucosal PD, resistance, and calculated short-circuit current. The ED_{50} of nicotine to H^+ secretion was 1.4 × 10^{-2} *M*. Identical doses of nicotine, when added under the conditions of high or low basal H^+ secretion, produced a greater percentage inhibition of the low H^+ secretory state.

Discussion. Olbe and Elwin (3) reported that the introduction of 0.1–0.5% nicotine into the antrum can elicit gastric acid secretion in dogs, probably by activating the release of antral gastrin. Cigarette smoking, however, had no effect on the response to sham feeding. Wilkinson and Johnston (4) have shown recently that cigarette smoking produces highly significant inhibition of acid

TABLE I. Effects of Nicotine on Basal H^+ Secretory Rate, Transmucosal PD, Resistance and Calculated Short-Circuit Current in Isolated Bullfrog Gastric Mucosa.[a]

Parameter	Control[b]	Nicotine (M)			
		6.0×10^{-3}	1.2×10^{-2}	2.4×10^{-2}	4.8×10^{-2}
H^+ rate (μeq/cm^2 • hr)	3.33 ± 0.15	2.71 ± 0.16[d]	1.45 ± 0.06[f]	0.36 ± 0.05[f]	0 ± 0[f]
PD (mV)	17.9 ± 1.3	24.0 ± 2.0[d]	35.1 ± 0.6[f]	29.0 ± 0.8[f]	50.6 ± 0.8[f]
Resistance (Ohm cm^2)	131 ± 5	139 ± 3	169 ± 9[e]	240 ± 2[f]	256 ± 3[f]
I_{sc}[c] (μA/cm^2)	137 ± 8	171 ±10[d]	209 ±10[f]	163 ± 4[d]	197 ± 2[f]

[a] Values during the 44–48-min period which was immediately followed by removal of nicotine are shown as mean ± SE of the mean. A given dose of nicotine was added to the nutrient side of the mucosa 24 min after the start of each experiment.

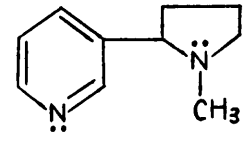
[b] Values during the corresponding period without nicotine.

[a,b] Each value for six experiments in six mucosae.

[c] Calculated short-circuit current (the ratio PD/resistance).

[d] $p < 0.05$; [e] $p < 0.01$; [f] $p < 0.001$ when compared with control (nonpaired t test).

and pepsin response to pentagstrin in man. "Smoking doses" of nicotine are considered to be 1–100 μg/kg iv (5); and monkeys, under certain circumstances, will self-administer 2000 μg/kg of nicotine intravenously (6). Since 5–40 μg/kg iv nicotine as well as tobacco smoke causes a rise in blood pressure which is frequently preceded by a transient small fall (7), the inhibitory action of tobacco smoke on gastric secretion is probably the results of the effects of nicotine on multiple sites in the organism. It is well known that nicotine exerts its action on a number of sites, both peripheral and central. For example, nicotine, when given intravenously, has been shown to cause a rise in blood pressure both in man and animals, mainly due to the stimulation of the sympathetic ganglia and the adrenal medulla together with discharge of catecholamines from various storage sites, by stimulation of the carotid and aortic chemoreceptors, and by a possible direct vasomotor stimulation (8). The present findings show that nicotine inhibits acid secretion with a concomitant rise in the transmucosal PD, resistance, and calculated short-circuit current. These effects of nicotine, including the reversibility, were qualitatively similar to those of SCN^-, CNO^-, NO_2^-, and NH_4^+ (9). From the results shown in Fig. 1 and Table I, the striking phenomena are that nicotine inhibits the H^+ transporting mechanism and the Cl^- transporting mechanism continues; in other words, there is a separation of the H^+ and Cl^- pump. Hogben (10) found that the short-circuit current is nearly equivalent to an active transport of Cl^- from the serosal to the mucosal side. It has also been shown that the calculated short-circuit current is approximately equal to the short-circuit current determined by the conventional technique (11). Since nicotine was found to increase the calculated short-circuit current, it seems that the Cl^- transport mechanism was activated by nicotine. LeFevre *et al.* (9) demonstrated that thiocyanate, cyanate, nitrite, and ammonium ions can inhibit acid secretion reversibly, and proposed that the inhibitory action depends upon the presence of a nitrogen atom with a pair of unshared electrons. In fact the structural formula of nicotine fits this as follows:



Preliminary experiments show that hexamethonium ($3 \times 10^{-2} M$) can not block the response to 6×10^{-3} M nicotine. Thus it seems likely that nicotine acts directly on the secretory cells. Although the gross pharmacological phenomena of nicotine action has been well investigated, the various events produced by the compound at cellular and subcellular levels must be further explored.

Summary. The effects of nicotine on H^+ secretion, transmucosal PD, and resistance were studied in isolated bullfrog gastric mucosa. It was found that nicotine produced a reversible dose-related inhibition of H^+ secretion, with an increase in transmucosal PD, resistance, and calculated short-circuit current. The results indicate that nicotine may selectively inhibit H^+ secretory mechanism.

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