PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 177, 447–454 (1984)

Hemodynamic Actions of Nicotine on the Canine Stomach (41971)

WIESLAW W. PAWLIK, ROBERT O. BANKS, AND EUGENE D. JACOBSON

Department of Physiology and Biophysics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Abstract. Acute effects of nicotine were determined in the circulation of the stomach in which measurements of gastric blood flow, systemic arterial and portal venous blood pressures, and the arteriovenous oxygen content difference were made. From these measurements gastric vascular resistance and oxygen consumption were calculated. Nicotine was infused for 10-min periods in two doses intraarterially (ia) and intravenously (iv) alone and following adrenergic receptor blockade with either propranolol or phentolamine or both agents. With ia nicotine alone, gastric blood flow and oxygen consumption increased. The effect of nicotine on blood flow was enhanced by prior treatment with phentolamine. The effects of nicotine on both blood flow and oxygen consumption were abolished in the presence of combined phentolamine plus propranolol. With iv nicotine alone, gastric blood flow, oxygen consumption, and arterial pressure increased transiently. Phentolamine enhanced the effect of nicotine on oxygen uptake and prevented the pressor response. Propranolol enhanced the pressor effect of nicotine but abolished the blood flow and oxygen consumption responses. Combined treatment with both adrenergic antagonists abolished nicotine effects on blood flow and oxygen consumption. These findings indicate that nicotine alters gastric hemodynamics and oxygen consumption during acute intravascular infusions of the drug. The vasodilator and metabolic effects of nicotine appear to be mediated via β-adrenergic receptors. These findings provide little basis for implicating a local ischemic mechanism in the ulcerogenic effects of nicotine on the stomach.

Cigarette smoking has been suspected as a causal factor in peptic ulcer disease in reports dating to the early part of this century (1). The mechanisms by which tobacco products predispose to peptic ulcers are uncertain, although increased gastric acid secretion has been proposed (2). Ulceration could also result from a decline in mucosal resistance to injury by acid gastric juice. One significant factor in retention of normal resistance to corrosive secretions is the adequacy of the mucosal circulation.

Nicotine is a major active alkaloid of tobacco and is usually taken into the body during smoking or chewing of tobacco products. Nicotine can reach the gastric mucosa as a bloodborne chemical or can contact the mucosa from the lumen after the substance has been swallowed.

Recently, we examined the effects of intravenously and intraarterially infused nicotine on the small intestine and found that the alkaloid prompted variable mesenteric hemodynamic responses which reflected mainly an indirect action of nicotine mediated by release of catecholamines (3). If nicotine were to evoke a localized ischemic hypoxia in the stomach, such an effect could contribute to the loss of mucosal resistance to injury by gastric juice and might explain the ulcerogenicity of cigarette smoking. Therefore, in the present study we have extended our investigation of this vasoactive agent to the stomach. We have evaluated the effects of nicotine on gastric hemodynamics and on oxygen consumption and have explored these effects during antagonism of α- and β-adrenergic receptors.

Methods. Experiments were performed on 54 dogs of either sex, weighing between 19 and 30 kg. The animals were fasted but allowed access to water for 24 hr before the onset of experiments. Anesthesia was induced by a slow intravenous (iv) injection of sodium pentobarbital (30 mg/kg). The animals were ventilated with room air using a positive pressure respirator (Harvard Apparatus, Millis, Mass.) and a cuffed endotracheal tube. A femoral artery was cannulated and connected to a strain-gauge transducer (Hewlett–Packard Co., Waltham, Mass.) for continuous monitoring of systemic arterial pressure on a re-

0037-9727/84 $1.50

corder (Hewlett–Packard) and a femoral vein was cannulated for periodic injection of anesthetic as needed.

A midline laparotomy was performed, following which the stomach and spleen were exposed and all nongastric branches of the splenic artery and vein were ligated. One polyethylene catheter was introduced retrogradely into a branch of the splenic artery for intra-arterial (ia) infusion of drugs or saline directly into the gastric vascular bed. Two splenic vein branches were also cannulated. One saline-filled catheter was inserted into the portal vein and connected to a pressure transducer for continuous measurement of pressure. Another catheter was introduced into one of the splenic veins draining the area supplied by the left gastroepiploic artery. This catheter was used to obtain venous blood from the stomach for perfusion through an arteriovenous oxygen content difference analyzer (AVOX System, San Antonio, Tex.). A part of the fundic portion of the stomach supplied by the left gastroepiploic artery was then isolated by clamping with a forceps, thereby forming a gastric pouch. We ligated the larger connections of the left gastroepiploic artery with other gastric arteries as well as all branches supplying the omentum in order to interrupt collateral vessels. A wide-bore, saline-filled, multihole polyethylene catheter was inserted into the lumen of the gastric pouch and connected to a pressure transducer for measurements of intraluminal gastric pressure. Next, the stomach was covered with a saline-soaked gauze and plastic wrap.

Gastric blood flow was determined by means of an electromagnetic blood flow probe (2.0–2.5 mm i.d.) placed around the splenic artery proximal to the infusion site. The probe was connected to a blood flow amplifier (Zepeda Instruments, Seattle, Wash.). Continuous recordings of mean blood flow were made on a polygraph (Hewlett–Packard). The electromagnetic flow probe was calibrated *in vitro* and a zero flow reference was established during the experiment by brief occlusion of the splenic artery distal to the flow probe. Gastric oxygen consumption was calculated as the product of simultaneously measured blood flow to the gastric pouch and the arteriovenous oxygen content difference. Gastric vascular resistance was calculated from the quotient of (arterial/venous) pressure/blood flow.

After administration of sodium heparin (6.0 mg/kg) the gastric venous catheter and a femoral arterial catheter were attached to a constant-flow pump (Raivin Instruments, Co., Woburn, Mass.) to permit gastric venous and femoral arterial blood to flow through separate cuvettes of the oxygen content difference analyzer at 7.0 ml/min. The blood was returned to the animal via a femoral vein catheter.

Circulatory parameters were allowed to stabilize. Drugs (nicotine, phentolamine, propranolol) were not infused until observed variables had been steady for 30 min. One of nine experimental protocols was then initiated. The design of these protocols appears in Table I. In each protocol a group of six dogs was studied. In the first four groups

TABLE I. DESIGN OF EXPERIMENTS WITH NICOTINE—CIRCULATORY STUDIES

Group	Route of administration	Dose of nicotine (μg/min-kg)[a]	Presence of antagonist drug	
			α (phentolamine)	β (propranolol)
I	ia	25		
II	ia	50		
III	ia	50	+	
IV	ia	50	+	+
V	iv	5		
VI	iv	20		
VII	iv	20	+	
VIII	iv	20		+
IX	iv	20	+	+

[a] All infusions of nicotine lasted 10 min.

nicotine was infused intra-arterially. In group I each dog was administered nicotine (Sigma, St. Louis, Mo.) into the left gastroepiploic artery at a dose of 25.0 μg/min-kg body wt for 10 min. During the smoking of one cigarette the smoker may absorb up to 3 mg nicotine (3). The corresponding dose of infused nicotine in a 20-kg dog would be 5 μg/min–kg over 10 min. In group II nicotine was infused at a dose of 50.0 μg/min-kg for 10 min. In group III an infusion of nicotine (50.0 μg/min-kg for 10 min) was performed within 5 min after α-receptor antagonism was attained with phentolamine (Regitine, Ciba-Geigy Co., Summit, N.J.). Phentolamine was administered for 10 min by a continuous intravenous infusion at a rate of 0.5 mg/min. This slow rate of administration was employed in order to minimize the depressor effect of phentolamine. In group IV we infused nicotine (50.0 μg/min-kg) within 5 min after administering α- and β-adrenergic receptor antagonist drugs. In this group, phentolamine was administered as previously described and blockade of β receptors was achieved with propranolol (Ayerst Laboratories, Inc., New York) using an intravenous dose of 0.5 mg/kg which was injected slowly after the administration of phentolamine.

In groups V–IX nicotine was infused intravenously. In group V the dose of nicotine was 5.0 μg/min-kg for 10 min. In group VI dogs received an infusion of nicotine at a dose of 20.0 μg/min-kg for 10 min. In group VII dogs received an infusion of nicotine (20.0 μg/min-kg) after administering the α-receptor antagonist, phentolamine (0.5 mg/min iv × 10 min). In group VIII dogs received a 10-min infusion of nicotine (20.0 μg/min-kg) for 10 min, after administering the β-receptor antagonist, propranolol. In group IX dogs received an infusion of nicotine (20.0 μg/min-kg for 10 min) after α- and β-adrenergic receptor blockade.

The effectiveness of adrenergic receptor antagonism with phentolamine and propranolol in anesthetized dogs was reported previously from our laboratory. We had found that the slow iv infusion of 5 mg phentolamine reduced the pressor response to iv norepinephrine (1 μg/kg) by 72% (3). Propranolol (0.5 mg/kg iv) effectively blocked the vasodilatory responses to ia isoproterenol (1.0 μg/min-kg) in canine mesenteric artery (4).

We also determined the effect of the adrenergic antagonists on both gastric blood flow and systemic arterial pressure by measuring these circulatory variables just before administering propranolol, phentolamine, or both drugs and, following cessation of drug infusion, before infusing nicotine.

The significance of changes in measured values from control was determined using the paired t test with a confidence limit of less than 5%.

Results. The control values in these nine groups of experiments were gastric blood flow, 65.4 ± 8.0 ml/min per 100 g tissue; arteriovenous oxygen difference, 3.5 ± 0.3 ml O_2/100 ml blood; gastric oxygen consumption, 2.4 ± 0.6 ml O_2/min per 100 g of tissue; mean systemic arterial pressure, 130 ± 7.0 mm Hg; portal venous pressure, 5.4 ± 0.6 mm Hg; and gastric vascular resistance, 2.7 ± 0.4 mm Hg/ml per min blood flow per 100 g tissue.

The effects of ia infusion of nicotine (25.0 and 50.0 μg/min-kg body wt—Groups I and II, respectively) on gastric blood flow, oxygen difference and uptake, and arterial pressure appear in Figs. 1 and 2. Infusion of the lower dose of nicotine induced significant increases in gastric blood flow (8.7 ± 2.0%) and in oxygen consumption (11.0 ± 1.5%). Infusion of the higher dose of nicotine induced significantly larger increases in blood flow (14.3 ± 2.0%) and oxygen consumption (16.5 ± 2.5%). The pattern of hyperemia observed with the higher dose of nicotine exhibited a transient peak response at 1 min followed by a stable but lesser hyperemia for the rest of the infusion period (Fig. 1). The increase in oxygen consumption was also evident at 1 min and did not vary significantly from that increase for the next 9 min (Fig. 1).

The effects of ia infusion of nicotine on gastric blood flow, oxygen extraction, and oxygen consumption were compared between nicotine alone (group II), nicotine after α-adrenergic blockade (group III), and nicotine after both α- and β-adrenergic blockade (group IV). These data appear in Fig. 3. Following α-adrenergic blockade with phentolamine, nicotine induced significant increases in gastric blood flow (32.5 ± 6%) and

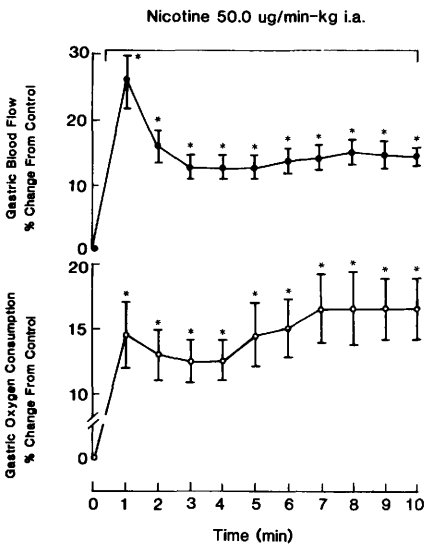

FIG. 1. Effects of nicotine on gastric blood flow and oxygen consumption during a 10-min period of close intraarterial infusion of the drug at a dose of 50 μg/min-kg body wt. An asterisk above a data point indicates a significant ($P < 0.05$) increase over the prenicotine control value.

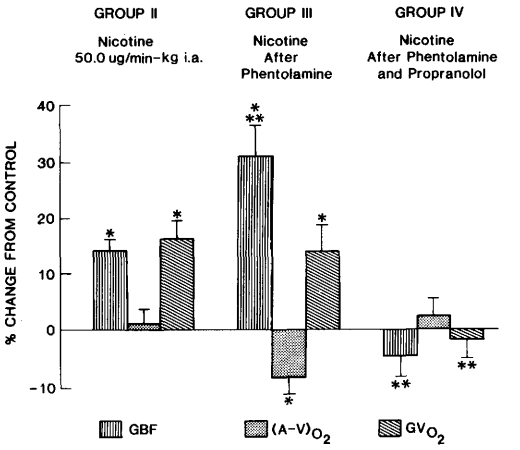

FIG. 3. A comparison of the effects of intraarterially infused nicotine at the 50 μg/kg-min dose alone and after pretreatment with either phentolamine or phentolamine + propranolol. A single asterisk above or below a bar indicates a significant change from its prenicotine control value; two asterisks below a bar indicate a significant difference from the value observed with nicotine alone but not significantly different from the prenicotine control value; three asterisks above the bar indicate a significant difference from both the prenicotine control value and the value observed with nicotine alone.

oxygen consumption (13 ± 4%), but significantly decreased (−9 ± 2%) the arteriovenous oxygen difference. The increase in blood flow

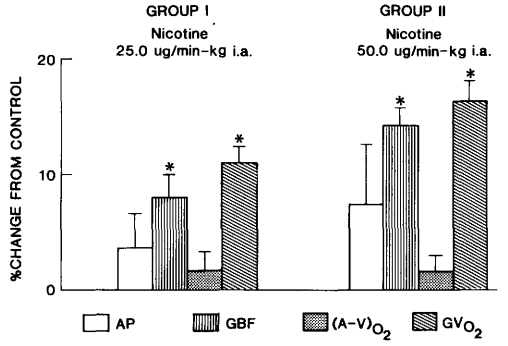

FIG. 2. A comparison of the effects of intraarterially infused nicotine at two doses on systemic arterial blood pressure (AP), gastric blood flow (GBF), the arteriovenous oxygen content difference $(A-V)O_2$ and calculated gastric oxygen consumption (GVO_2). Group numbers refer to their designation and description under Methods. The values were obtained at 10 min after start of the infusion and are expressed as a percentage change from the values in the last minute before starting the infusion. An asterisk above a bar indicates a significant ($P < 0.05$) increase over the control value.

was significantly greater than that observed with nicotine alone (group II). With combined α- and β-adrenergic receptor blockade, nicotine did not affect blood flow or oxygen related variables.

Intravenously administered nicotine (5.0 μg/min-kg) induced significant but small (+10% or less) increases in both gastric blood flow and systemic arterial pressure, effects which appeared late in the 10-min infusion period (group V). Similarly, there was a small (−8%) decrease in oxygen consumption noted by the tenth minute of intravenous drug infusion.

The iv infusion of a higher dose (20.0 μg/min-kg) of nicotine was examined before (group VI) and during either α-adrenergic blockade (group VII), β-adrenergic blockade (group VIII), or combined α- and β-adrenergic blockade (group IX). Results of these experiments appear in Fig. 4 (gastric blood flow), Fig. 5 (oxygen consumption), and Figure 6 (arterial blood pressure). Without adrenergic blockade, iv nicotine significantly increased blood flow with a peak value (46.0 ± 12%) reached at 4 min (Fig. 4). At the end

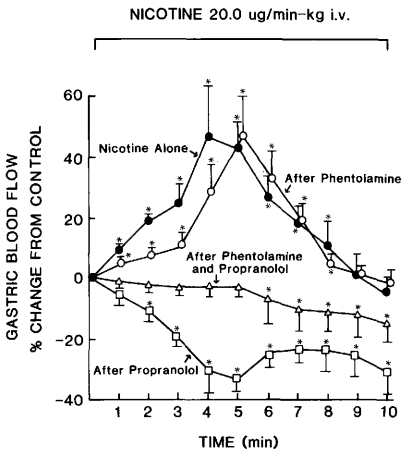

FIG. 4. A comparison of the effects of a 10-min intravenous infusion of nicotine on gastric blood flow in four groups of animals: nicotine alone (group VI), nicotine after phentolamine (group VII), nicotine after propranolol (group VIII), and nicotine after combined treatment with both phentolamine and propranolol (group IX). An asterisk above or below a data point indicates a significant difference from the last prenicotine control value.

of the 10-min period of infusion, flow had returned to its control value. Oxygen consumption increased slightly at the beginning of nicotine infusion and then decreased significantly during the sixth and seventh minute of infusion, returning to the control value by the end of the infusion period (Fig. 5). Arterial blood pressures were significantly increased during the infusion period (Fig. 6). After α-adrenergic blockade with phentolamine nicotine induced the same response in blood flow, despite a reduced arterial pressure. Gastric oxygen consumption was increased significantly during the infusion of nicotine in α-adrenergic-blocked animals and arterial blood pressure was significantly decreased. During β-adrenergic blockade nicotine decreased blood flow and oxygen consumption while increasing arterial pressure. After α-plus β-adrenergic blockade nicotine significantly decreased blood flow and oxygen consumption but significantly increased arterial pressure.

Intragastric pressure recordings obtained during either ia or iv infusions of nicotine

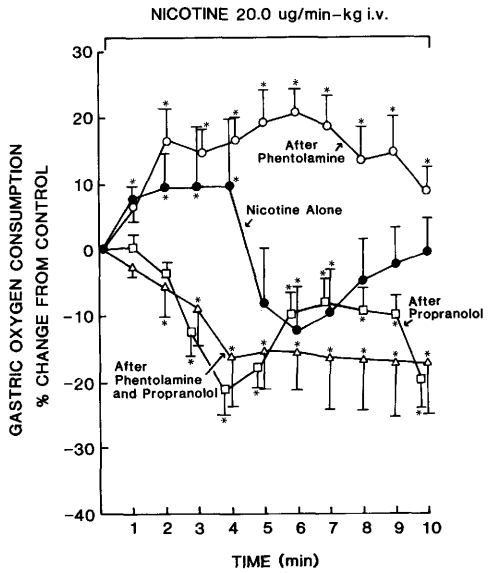

FIG. 5. A comparison of the responses to a 10-min intravenous infusion of nicotine on gastric oxygen consumption in four groups of animals: nicotine alone (group VI), nicotine after phentolamine (group VII), nicotine after propranolol (group VIII), and nicotine after combined treatment with both phentolamine and propranolol (group IX). An asterisk above or below a data point indicates a significant difference from the last prenicotine control value.

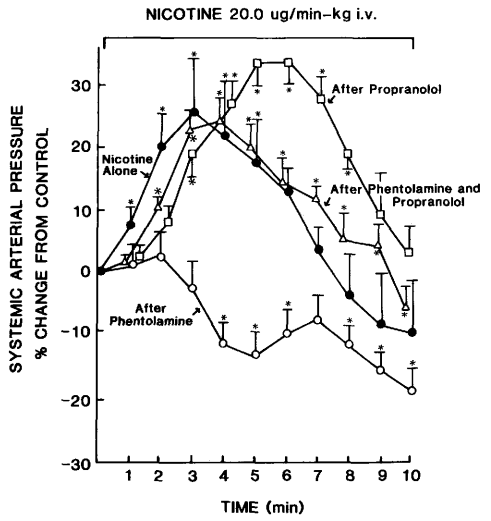

FIG. 6. A comparison of the responses to a 10-min intravenous infusion of nicotine on systemic arterial blood pressure in four groups of animals: nicotine alone (group VI), nicotine after phentolamine (group VII), nicotine after propranolol (group VIII), and nicotine after combined treatment with both phentolamine and propranolol (group IX). An asterisk above or below a data point indicates a significant difference from the last prenicotine control value.

yielded inconclusive results. Thus, ia nicotine (50 μg/min-kg) increased pressure at 1 min but decreased pressure below control values from the fourth to tenth minute of observation. On the other hand, iv nicotine (20 μg/min-kg) decreased intragastric pressure from the second to seventh minute of observation. Although these changes were significant, the magnitude of change was only a few millimeters Hg in either direction.

Finally, we determined the effect of propranolol, phentolamine, and the combination of agents on gastric blood flow and systemic arterial blood pressure before nicotine was infused. Results in Table II indicate that propranolol had minimal effects on either measurement, whereas phentolamine decreased arterial blood pressure by 18% and blood flow by 22%. The combination of both drugs reduced these measurements by 19 and 28%, respectively.

Discussion. Administration of nicotine either by intra-arterial or by intravenous routes evoked significant circulatory and metabolic responses in the stomach. During intra-arterial infusion we observed vasodilation (calculated gastric vascular resistance decreased) and an increased consumption of oxygen. These responses to ia nicotine were not inhibited by the α adrenergic antagonist, phentolamine, but were abolished in animals pretreated with the β adrenergic antagonist, propranolol, in combination with phentolamine. Intravenously administered nicotine also increased blood flow to the stomach, as well as gastric oxygen consumption; however, the hyperemia was accompanied by a systemic pressor response and no change in calculated vascular resistance, indicating no direct vasodilator response to nicotine. α-Adrenergic blockade alone prevented the systemic pressor response to intravenous nicotine and converted nicotine into a gastric vasodilator agent. Surprisingly, combined α and β antagonism did not prevent the pressor response to nicotine. Either β-adrenergic blockade or combined β- and α-adrenergic blockade prevented the increase in blood flow observed with intravenous nicotine.

Nicotine increased oxygen consumption within the stomach. This action of the drug appears to be secondary to its hemodynamic effect rather than as a result of an increase in gastric metabolism per se, since the arteriovenous oxygen content difference was not increased by nicotine. Similar effects have been reported with prostacyclin in the mesenteric circulation (5).

In a previous investigation of the canine small intestine, we observed that intravenously administered nicotine prompted an initial increase in blood flow to the gut accompanied by a pressor effect and no decrease in vascular resistance (3). In the same study large doses of intraarterial nicotine caused an increase in mesenteric blood flow which could be prevented by ganglion blockade. Furthermore, isometrically contracted strips of mesenteric smooth muscle were not contracted further by nicotine (10^{-3} *M*) in the presence of phentolamine (10^{-5} *M*). In preliminary investigations in the renal circulation we have found that intraarterial administration of nicotine did not alter renal

TABLE II. EFFECTS OF ADRENERGIC ANTAGONISTS ON HEMODYNAMIC PARAMETERS IN THREE SERIES OF DOGS

	Control value	Post-drug value
Propranolol (*N* = 6)		
Gastric blood flow (ml/min-100 g)	68 ± 4	64 ± 4*
Systemic arterial pressure (mm Hg)	142 ± 8	137 ± 7*
Phentolamine (*N* = 14)		
Gastric blood flow (ml/min-100 g)	64 ± 4	52 ± 4**
Systemic arterial pressure (mm Hg)	125 ± 8	102 ± 10**
Both drugs (*N* = 9)		
Gastric blood flow (ml/min-100 g)	84 ± 8	60 ± 4**
Systemic arterial pressure (mm Hg)	129 ± 8	101 ± 8**

* Significant at *P* less than 0.05 by nonparametric comparison.
** Significant at *P* less than 0.05 by a paired *t* test.

blood flow but did increase renal excretion of solutes, urine flow rate and glomerular filtration (6). The effects of nicotine on renal excretory parameters were abolished either by bilateral adrenalectomy or by prior adminstration of propranolol. Thus, it appears that the actions of nicotine on circulatory and functional activities in three separate organs are predominantly indirect and involve both receptors of the adrenergic system. Taken together, our findings in the aforementioned investigations also suggest that nicotine stimulates both peripheral sympathetic nerves and adrenal medullary release of catecholamines, especially epinephrine, since β-adrenergic activity was so prominent in the stomach after nicotine.

Stimulation of adrenergic receptors during infusion of nicotine would be expected to alter gastric blood flow. Electrical stimulation of gastric sympathetics (7) or administration of norepinephrine (8) constricts the gastric circulation. Isoproterenol has a vasodilator effect on the stomach unless the dose is such as to inhibit secretion, in which case gastric blood flow will diminish (8).

Another indirect effect of nicotine which could reduce gastric blood flow is the action of the alkaloid on the posterior pituitary gland. Nicotine stimulates cervical parasympathetic nerves to the gland and causes the release of vasopressin (9). In large doses vasopressin constricts the gastric circulation (8).

In the present investigation we utilized two routes of administration of nicotine in order to both identify the responses to nicotine and determine whether the effects of the drug were mediated directly or indirectly. The close intraarterial infusion of nicotine evoked a significant increase in blood flow to the stomach without raising arterial pressure significantly (Fig. 2). This finding suggested the possibility of direct vasodilator action of the drug. However, the hyperemia was abolished by antagonizing β-adrenergic receptors with propranolol prior to administering nicotine into the gastric circulation, implying that the nicotine effect is more likely an indirect event involving the adrenergic system. The use of the ia route of administration of nicotine also imposed the burden of an excessive concentration of the drug on the gastric circulation before significant changes in blood flow or oxygen consumption were evident. Thus, the cumulative dose of nicotine per kilogram body weight associated with an ia infusion at a rate of 25 μg/min-kg for 10 min in a 25-kg dog is equivalent to that amount of the drug caused by smoking five cigarettes in 10 min. Furthermore, by delivering the drug directly into the splenic artery (which bears less than 5% of the cardiac output) the concentration of nicotine is further increased in the gastric circulation compared with the concentration which would result from an iv infusion. As a result of this limitation, we also utilized an intravenous route of administration of nicotine to obtain a drug concentration in the gastric circulation closer to that experienced by the cigarette smoker. The disadvantage of the iv route is that the indirect effects of nicotine are likely to be more prominent and to occur before direct effects of the alkaloid become evident, thereby even masking the direct effects. Despite the differences imposed by these two routes of administration, nicotine prompted the same acute events in the stomach, namely an increase in blood flow and oxygen consumption. Moreover, both responses to nicotine in both groups of dogs were prevented by antagonizing β-adrenergic receptors.

Use of the adrenergic receptor antagonists was not without significant effects on the circulation (Table II). Phentolamine alone or in combination with propranolol lowered arterial pressure and blood flow to the stomach by one-fifth or more, whereas propranolol alone caused minimal changes in either variable. Interestingly, propranolol was the most effective agent in preventing the gastric circulatory and metabolic responses to nicotine.

Our findings do not implicate gastric ischemia in the mechanism of nicotine ulcerogenesis. When administered directly into the gastric arteries, nicotine evoked predominately a β-adrenergic response, namely vasodilation, with an increase in both mucosal blood flow and oxygen consumption. When administered intravenously the drug still increased gastric blood flow and oxygen consumption. These results do not preclude a damaging effect of nicotine on mucosal resistance to gastric juice, but such an effect, if it exists, does not appear to operate via an

acute vasoconstrictor response to the agent. Our findings deal only with acute responses to nicotine and the chronic response to the drug might differ.

These investigations were supported by a grant from the Smokeless Tobacco Research Council, Inc.

1. Barnett CW. Tobacco smoking as a factor in the production of peptic ulcer and gastric neuroses. Boston Med Surg J **197**:457–459, 1927.
2. Murthy SNS, Dinoso VP Jr, Clearfield AR, Chevy, WY. Simultaneous measurement of basal pancreatic and gastric acid secretion, plasma gastrin and secretin during smoking. Gastroenterology **73**:758–761, 1977.
3. Gallavan RH Jr, Tsuchiya Y, Jacobson ED. The effects of nicotine on canine intestinal blood flow and oxygen consumption. Amer J Physiol **246**:G195–G203, 1984.
4. Walus KM, Fondacaro JD, Jacobson ED. Effects of adenosine and its derivatives on the canine intestinal vasculature. Gastroenterology **81**:327–334, 1981.
5. Gallavan RH Jr, Fondacaro JD, Jacobson ED. Intestinal blood flow and oxygen consumption. Proc Soc Exp Biol Med **174**:74–78, 1983.
6. Banks RO, Pawlik WW, Jacobson ED. Effects of nicotine on renal function. Fed Proc **43**:720 (Abstract), 1984.
7. Reed JD, Sanders DJ, Thorpe J. The effect of splanchnic nerve stimulation on gastric acid secretion and mucosal blood flow in the anesthetized cat. J Physiol **214**:1–13, 1971.
8. Jacobson ED, Linford RH, Grossman MI. Gastric secretion in relation to mucosal blood flow studied by a clearance technique. J Clin Invest **45**:1–13, 1966.
9. Cadnapaphornchai P, Boykin JL, Berl T, McDonald KM, Schrier RW. Mechanism of effect of nicotine on renal water excretion. Amer J Physiol **227**:1216–1220, 1974.

Received May 4, 1984. P.S.E.B.M. 1984, Vol. 177.
Accepted September 5, 1984.