Intracerebroventricularly Administered Nicotine Inhibits Vagally Stimulated Gastric Acid Output in Rats by Activating the Central Sympatho-Adrenal Outflow

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Abstract—The effect of intracerebroventricularly (i.c.v.) administered nicotine was investigated in urethane-anesthetized rats. I.c.v., but not intravenously, administered nicotine (300 and 600 nmole/animal) inhibited the increase in gastric acid output induced by electrical stimulation of the vagus nerve. This antisecretory effect of nicotine was abolished by combined pretreatment with adrenalectomy and 6-hydroxydopamine (50 mg/kg, i.v., 3 days before). I.c.v.-administered nicotine also raised the blood levels of catecholamines. These observations suggest that i.c.v. administered nicotine leads to excitation of the central sympathoadrenomedullary outflow and inhibits gastric acid output induced by stimulation of the vagus nerve.

Central administration of nicotine has dual effects on gastric acid output, increase in the basal gastric acid output (1, 2) and inhibition of the stimulated gastric acid output by 2-deoxy-D-glucose or electrical stimulation of the lateral hypothalamic area (3, 4). We now report another central inhibitory effect of nicotine, in a large dose, on gastric acid output.

Male Wistar rats weighing 350–400 g were deprived of food for 16 hr but were allowed free access to drinking water. These rats were anesthetized with 10% urethane solution (1.2 g/kg, i.p.). Through a cervical incision, the trachea and esophagus were exposed and respectively cannulated and ligated. Bilateral vagus were carefully separated from the carotid arteries and cut at the cervical portion. The peripheral end of the left side vagus nerve was placed on platinum ring electrodes and covered with cotton soaked in paraffin oil. The cervical incision was then sutured. The femoral vein was cannulated for infusion of saline or a drug. The abdomen was opened by a midline incision; a round-tip polyethylene cannula (3.5 cm in length and 0.4 cm in diameter) was inserted into the stomach via an incision in the duodenum. The cannula was held in place by two ligatures around the duodenum, one at the oral site and the other at the anal site of the duodenal incision, and the abdominal incision was sutured. The animal was mounted in a stereotaxic apparatus, and a stainless steel needle through which the drug solutions (10 μl) were applied i.c.v. was inserted into the right ventricle (AP 7.0; L 1.1; H 4.0 below the surface of the brain) according to the Brain Atlas by König and Klippel (5).

After washout of the stomach with saline, 2.0 ml of gastric solution prewarmed at 38°C was instilled. The solution, replaced at 15-min intervals, consisted of a 1/5 (v/v) mixture of glycine and mannitol adjusted to 300 mOsmol and pH 3.5 by the addition of 0.1 N HCl, according to Blair et al. (6). After stabilization of the basal acid output, gastric acid output was stimulated by continuous electrode stimulation of the left vagus nerve, the stimulus parameters being square-wave pulses of 0.5 msec duration, at 3 Hz, supramaximal intensity (0.5 mA). These conditions were the same as previously reported (7, 8). Gastric acid output was determined by titration with 0.01 N NaOH and expressed as μEq/15 min, as shown in previous papers.
Nicotine was dissolved in saline solution. In the control animals, 0.3 M sodium phosphate buffer (pH 3.2) was applied in the same volume. 6-Hydroxydopamine hydrochloride (6-OHDA) (50 mg/kg), dissolved in saline containing 0.5% W/V ascorbic acid, was administered through the dorsal penile vein of an ether-anesthetized animal, 3 days before the experiments.

Catecholamines in the blood were assayed as follows: To a 2 ml blood sample was added 3 ml of 0.5 M perchloric acid, 2 mg of EDTA Na and 2 mg of Na2S2O4. After centrifugation (20,000 g for 15 min at 4°C), the supernatant was adjusted to pH 6.5 by addition of 1 M K2HPO4 and was then passed through the column (5 x 50 mm) of Amberlite CG-50. The column was washed with 4 ml of 0.1 M phosphate buffer (pH 6.5), 2 ml of 1 mM EDTA, 1 ml of H2O and 1.2 ml of 0.5 M HCl. Catecholamines in the blood, eluted with 3.4 ml of 0.5 M HCl from Amberlite CG-50, and catecholamines in the tissue, extracted by perchloric acid, were absorbed onto the aluminum oxide (9) and assayed electrochemically by high-performance liquid chromatography.

I.c.v.-administered nicotine (300 and 600 nmole/animal) did not affect the basal gastric acid output. When the left vagus nerve was continuously stimulated at 3 Hz, the gastric acid output rapidly increased and reached a steady level within 60 min. This steady level was maintained for over 60 min by continuous stimulation of the vagus nerve (Fig. 1A). Thus, nicotine (300 and 600 nmole/animal) or vehicle was administered at 60 min after the start of vagal stimulation. I.c.v.-administered nicotine dose-dependently reduced the vagally stimulated gastric acid output: percent acid output with nicotine at the dose of 300 and 600 nmole/animal at the 8th 15-min collection period were 73.3% (data not shown) and 39.1% (Fig. 1A), respectively. These inhibitory effects persisted for over 60 min.

We reported that a small dose of nicotine given i.c.v. (100 nmole/animal) and microinjected into an area of the dorsal motor nucleus of the vagus inhibited the increase in gastric acid output induced by the administration of 2-deoxy-D-glucose and by electrical stimulation of the lateral hypothalamic area (3, 4). These inhibitory effects of nicotine were abolished by administration of both reserpine (i.v.) and phentolamine (i.c.v.).

Central noradrenergic inhibitory mechanisms regulate gastric functions; inhibition of central parasympathetic outflow by a noradrenergic mechanism results in decreased gastric acid output (10). Therefore, it was considered that nicotine (i.c.v.) activated the noradrenergic neuron system in the brain and inhibition of the central parasympathetic outflow ensued. However, in the present study, the nicotine (i.c.v.)-induced inhibition of the vagally stimulated acid output was not mediated by attenuation of the central parasympathetic outflow, since the cervical vagus nerves had been bilaterally cut beforehand.

As related to the peripheral effect of nicotine on gastric acid output, a large dose of this alkaloid (i.v.) (50 μg/kg per min, for 30 min; 9.25 μmole/kg) inhibits acid output by peripheral activation of the sympathoadrenomedullary system in cases of stimulation of the vagus nerve (8). In the present study, intravenous administration of this alkaloid (600 nmole/animal) has no inhibitory effect on the gastric acid output (Fig. 1B). Thus, the site of action of the i.c.v.-administered nicotine was central and not peripheral after leakage into the systemic circulation.

Electrical stimulation of the greater splanchnic nerve (branching out to the adrenal glands and forming a synapse with the gastric sympathetic nerve) activates the adrenal medulla and gastric sympathetic nerves, thereby inhibiting the vagally stimulated gastric acid output through activation of the adrenergic alpha-adrenoceptors in the stomach (7). Therefore, in the next series of experiments, we examined whether or not the i.c.v.-administered nicotine centrally activates the sympathoadrenomedullary system, based on preliminary observations that the present nicotine (i.c.v.)-induced inhibition was abolished by phentolamine (1 mg/kg, i.v.). (data not shown). Three days after the administration of 6-OHDA (50 mg/kg, i.v.), norepinephrine content in the
Fig. 1. Effects of nicotine on the vagally stimulated gastric acid output. (A) Intracerebroventricularly (i.c.v.) administered group. (B) Intravenously administered group. (C) i.c.v.-administered group pre-treated with bilateral adrenalectomy and 6-OHDA. Sixty min after start of vagal stimulation, nicotine (500 nmole/animal) or vehicle was administered. 6-OHDA (50 mg/kg) was intravenously administered 3 days before initiation of the experiments. (□) vehicle-administered control rats, (○) nicotine-administered rats. Values at different collection periods are expressed as a percentage of the value obtained at the 5th 15-min collection period. *P<0.05 (statistical difference was compared with the corresponding values of the control rats, using Student's t-test). Mean±S.E. of four rats. Values of the acid output at the 5th 15-min collection period were 64.2±9.0 μEq/1 5 min for group A (n=8), 75.0±5.3 μEq/15 min for group B (n=8) and 72.0±6.8 μEq/15 min for group C (n=8), respectively.

Stomach was reduced to 11.7% of the control level, while contents of catecholamines in the brain and adrenals remained unchanged. Therefore, adrenalectomy was performed in the 6-OHDA-pretreated animals to avoid the influence of circulating cate-
In conclusion, a large dose of i.c.v.-administered nicotine increases the central sympatho-adrenomedullary outflow and thus the vagally stimulated gastric acid output is inhibited.

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References