Centrally Applied Nitric Oxide Donors Inhibit Vagally Evoked Rat Gastric Acid Secretion: Involvement of Sympathetic Outflow

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ABSTRACT—Intracerebroventricularly (i.c.v.) administered nitric oxide (NO) donors, 3-morpholinosydnonimine (SIN-1) (100–500 µg/animal) and sodium nitroprusside (SNP) (100–250 µg/animal) dose-dependently inhibited the rat gastric acid secretion evoked by vagal stimulation at 3 Hz. Furthermore, the inhibitory effect of SIN-1 (250 µg/animal) was more marked and its onset was more rapid than that of SNP (250 µg/animal). The SIN-1 (250 µg/animal)-induced antisecretory effect was abolished by both splanchnicotony and phentolamine (5 mg/kg, i.m.), and also by indomethacin (500 µg/animal, i.c.v.). These results suggest that i.c.v. administered NO donors inhibit vagally evoked gastric acid secretion by activation of central sympathetic outflow. Central prostaglandin is probably implicated in this NO-mediated antisecretory effect.

Keywords: Gastric acid secretion, Nitric oxide, Sympathetic nervous system

We have reported that intracerebroventricularly (i.c.v.) administered interleukin-1β (IL-1β) inhibits the vagally evoked rat gastric acid secretion by activation of central sympathetic outflow and this cytokine-induced antisecretory effect was abolished by i.c.v. administered indomethacin, a cyclooxygenase inhibitor (1). A successive study demonstrated that i.c.v. administered IL-1β elevated plasma noradrenaline (NA) levels, and this effect was abolished by intracerebroventricular pretreatment with indomethacin and also with a nitric oxide (NO) synthase inhibitor and an NO scavenger (2). These evidence suggest that prostaglandin (PG) and NO in the brain play roles in central regulation of sympathetic outflow.

NO is formed in the brain as well as in the vascular endothelium and participates in various brain functions such as long-term potentiation in the hippocampus (3). At subcellular levels, NO activates not only guanylate cyclase, but also cyclooxygenase (4). We have reported that centrally administered PGE2 induces sympathetic outflow by activation of prostanoid EP3 receptors and inhibits vagally evoked rat gastric acid secretion (5). In the present study, therefore, we examined whether i.c.v. administered NO donors, sodium nitroprusside (SNP) and 3-morpholinosydnonimine (SIN-1), inhibit the vagally evoked rat gastric acid secretion by activation of central PG-mediated sympathetic outflow.

Male Wistar rats weighing 350–400 g were deprived of food for 16 hr but were allowed free access to tap water. Details of the experimental procedures were as described in our previous papers (1). Briefly, under urethane anesthesia (1.2 g/kg, i.p.), the femoral vein was cannulated for infusion of saline (1.5 ml/hr). Bilateral vagus nerves were cut at the cervical level, the peripheral end of the left side vagus nerve was placed on platinum ring electrodes, and then the cervical incision was sutured. The abdomen was opened by a midline incision, and a round-tip polyethylene cannula was inserted into the stomach via an incision in the duodenum. In some animals, bilateral greater splanchnic nerves that contain the gastric sympathetic preganglionic nerve were cut just beneath the diaphragm. After closing the abdominal incision, the animal was placed in a stereotaxic apparatus throughout the experiments for intracerebroventricular administration of test substances.

One hour was allowed to elapse before the start of experiments for stabilization of the basal acid secretion. Two milliliters of artificial gastric solution prewarmed at 38°C was instilled and replaced at intervals of 15 min. This solution consisted of a 1/5 (v/v) mixture of glycine and mannitol adjusted to 300 mOsmol and pH 3.5. The gastric acid secretion was elicited by continuous electrical stimulation of the left vagus nerve with square-wave pulses of 0.5-msec duration, at 3 Hz and supramaximal intensity (1 mA). Gastric acid secretion was determined...
by titration with 0.01 N NaOH to pH 7.0 and expressed as pEq/15 min. In some experiments, phentolamine (5 mg/kg) or propranolol (5 mg/kg) dissolved in saline was administered intramuscularly (i.m.) 30 min before the start of vagal stimulation.

NO donors, 3-morpholinosydnonimine (Cayman Chemical Co., Ann Arbor, MI, USA) and sodium nitroprusside (Sigma, St. Louis, MO, USA) and water-soluble indomethacin sodium trihydrate (Merck Sharp & Dohme, Rahway, NJ, USA) were dissolved in saline and administered in a volume of 10 µl into the lateral cerebral ventricle with a stainless steel cannula (0.35 mm outer diameter) at coordinates AP −0.8 mm from the bregma, L 1.5 mm from the midline, and H 4.0 mm below the surface of the brain according to the rat brain atlas of Paxinos and Watson (6).

Results were expressed as the mean ±S.E.M. Statistical analyses were performed by Student’s t-test (Table 1) or one-way analysis of variance (ANOVA) in which case Bonferroni’s correction for comparing a control to all other means was used as a posterioric test (Fig. 1). P values of less than 0.05 were taken to indicate significance.

The mean basal acid secretion was 3.3±0.5 pEq/15 min (n=25). Continuous electrical stimulation of the vagus nerve at 3 Hz evoked an increase in gastric acid secretion that lasted more than 60 min (Fig. 1). The mean evoked acid secretion 60 min after the start of the stimulation was 82.8±5.8 pEq/15 min (n=25). Because of the relatively large individual variations in these levels, the effect of test substances on acid secretion was expressed as a percentage of the value obtained immediately before intracerebroventricular administration of NO donors. Intracerebroventricular administration of SIN-1 (100, 250 and 500 µg/animal) reduced the vagally evoked gastric acid secretion in a dose-dependent manner (Fig. 1a). On the other hand, its administration by the intravenous route (500 µg/animal) was without effect (data not shown). Intracerebroventricular administration of SNP (100 and 250 µg/animal) also reduced the vagally evoked gastric acid secretion; however, the onset of its inhibitory effect was slower than that of SIN-1 (Fig. 1b). While SIN-1 spontaneously liberates NO in solution (7), SNP is stable in solution and liberates NO by metabolism in vascular smooth muscle (8). In the present study, the low potency of SNP for inhibition of acid secretion is probably due to slow liberation of NO in the brain.

In the following experiments, we examined the effects of splanchnicotomy, adrenoceptor blocking agents (phentolamine and propranolol), and indomethacin on the SIN-1-induced inhibition of gastric acid secretion (Table 1). In the splanchnicotomized animals treated with vehicle (10 µl saline, i.c.v.), continuous electrical stimulation of the vagus nerve at 3 Hz rapidly increased gastric acid secretion, and this increase lasted more than 60 min. Such a constant and long lasting increase in the vagally evoked acid secretion was similarly observed in the animals pretreated with phentolamine (5 mg/kg, i.m.), propranolol (5 mg/kg, i.m.) or indomethacin (500 µg/animal) (9). Displacement of the vagal nerve in the neck by placing a pad of zymosan (10 mg) for 30 min before the start of vagal stimulation did not significantly alter the response to vagal stimulation, suggesting that the vagal afferent pathway is not involved in the inhibitory effect of NO donors. In the splanchnicotomized animals pretreated with phentolamine and propranolol (5 mg/kg, i.m.), continuous electrical stimulation of the vagus nerve at 3 Hz rapidly increased gastric acid secretion, and this increase lasted more than 60 min. Such a constant and long lasting increase in the vagally evoked acid secretion was similarly observed in the animals pretreated with phentolamine (5 mg/kg, i.m.), propranolol (5 mg/kg, i.m.) or indomethacin (500 µg/animal) (9).
pl/animal, i.c.v.), and successively received vehicle (10 μl saline, i.c.v.) alone. The inhibitory effect of SIN-1 (250 pg/animal, i.c.v.) on the vagally evoked acid secretion was abolished by the following pretreatments: splanchnicotomy, phentolamine and indomethacin, while pretreatment with propranolol was without effect.

Continuous electrical stimulation of the vagus nerve (3 Hz, 0.5 msec) was started at -60 min. 3-Morpholinosydnonimine (SIN-1) (250 pg/animal) or vehicle (10 μl saline,) was administered intracerebroventricularly (i.c.v.) at 0 min. Phentolamine (5 mg/kg, i.m.), propranolol (5 mg/kg, i.m.) or indomethacin (500 pg/animal, i.c.v.) was administered at -90 min. The actual values of the evoked gastric acid secretion at 0 min were 99.1 ±9.6 μEq/15 min for splanchnicotomized rats (n=8), 88.7±7.0 μEq/15 min for phentolamine-pretreated animals (n=8), 96.4±3.1 μEq/15 min for propranolol-pretreated animals (n=8) and 79.3±7.7 μEq/15 min for indomethacin-pretreated rats (n = 8). N, number of experiments; a), Significantly different (P<0.05) from the vehicle-treated control.

Histochemical studies have demonstrated that NO synthase is widely distributed in the central nervous system, especially in the cerebellum, hippocampus, striatum and hypothalamus (9). Several independent lines of evidence suggest that NO is involved in the release of hypothalamic peptides such as corticotropin-releasing hormone (10, 11) and vasopressin (12). The hypothalamus is also an important autonomic center regulating central sympathetic outflow (13). In the present study, i.c.v. administered SIN-1 inhibited vagally evoked gastric acid secretion, while its administration by the peripheral route was without effect.

In summary, we demonstrate here that i.c.v. administered NO donors inhibited vagally evoked gastric acid secretion by activation of central sympathetic outflow. Central PG is probably implicated in NO donor-mediated antisecretory effects.

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