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IMPORTANCE OF VAGAL INPUT IN MAINTAINING GASTRIC TONE IN THE DOG

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SUMMARY

1. Using a gastric barostat to quantify variations in gastric tone, we had previously demonstrated that food ingestion or intestinal nutrient perfusion induces gastric relaxation. These data suggested a basal tonic contraction of the stomach during fasting.

2. To determine the role of vagal input in maintaining fasting gastric tone, we prepared two chronic canine models, either isolating both cervical vagal trunks in a cutaneous tunnel or including the supradiaphragmatic vagi within an implanted cooling jacket. In the fasted conscious dogs, we then studied the effect, on gastric tone, of acute and reversible vagal blockade by cooling.

3. Cervical vagal cooling produced a reversible gastric relaxation and increased the heart rate. Supradiaphragmatic vagal cooling produced a similar gastric relaxation without the cardiac effect.

4. Adrenergic blockade did not change either the base-line gastric tone or the cooling-induced relaxation. Adrenaline decreased gastric tone, but vagal cooling still produced a significant relaxation.

5. Atropine alone or combined with adrenergic antagonists produced a gastric relaxation that was not further increased by vagal cooling. Bethanechol increased gastric tone, an effect unchanged by vagal cooling.

6. We conclude that gastric tone during fasting is maintained by a cholinergic input, which is vagally mediated at both the cervical and the supradiaphragmatic levels.

INTRODUCTION

The walls of the proximal portion of the stomach have the particular ability to maintain a tonic muscular contraction, that is, gastric tone (Morgan, Muir & Szurszewski, 1981). Variations in gastric tone are instrumental in achieving the reservoir function of the stomach by regulating both gastric accommodation and gastric emptying (Debas, Yamagishi & Dryburgh, 1977; Jahnberg, 1977; Kelly, 1980). Unfortunately, the mechanisms regulating gastric tone are poorly characterized, mainly because of the methodologic problems involved in measuring gastric tone *in vivo*.

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In our laboratory, we have developed a gastric barostat that can quantify variations in gastric tone and have established the physiological patterns of gastric tonic activity in the dog (Azpiroz & Malagelada, 1985*a*) and in man (Azpiroz & Malagelada, 1985*c*). Under basal conditions (conscious, fasting and resting), the upper gut in dog and in man exhibits a cyclic interdigestive motor pattern, involving also the tonic activity of the stomach (gastric tone). This interdigestive activity pattern in the upper gut, however, can be modified by various stimuli, be they physiological (that is, food ingestion) (Code & Marlett, 1975) or noxious (that is, stress) (McRae, Younger, Thompson & Wingate, 1982; Valori, Parnham, Patrick, Raiman & Wingate, 1983), or by experimental manipulations (that is, gastric distension, anaesthesia and surgery) (Smith, Kelly & Weinshilboum, 1977; Azpiroz & Malagelada, 1984; Diamant, Scott & Davison, 1985). Gastric tone, in particular, can be substantially reduced (gastric relaxation) by food ingestion (Azpiroz & Malagelada, 1985*a*) or intestinal nutrient perfusion (Azpiroz & Malagelada, 1985*b*), suggesting that the stomach maintains a tonic contraction during fasting. However, whether this high level of gastric tone during fasting is intrinsic or consequent to an extrinsic neural input remains unknown. The existence of a tonic excitatory vagal input to the stomach has been proposed (Roman & Gonella, 1981; Miolan & Roman, 1984). In conflict with this postulate, vagotomy in previous studies has been consistently reported to increase gastric tone (Harper, Kidd & Scratcherd, 1959; Martinson, 1965; Jansson, 1969; Martinson, 1975; Jahnberg, 1977; Andrews & Lawes, 1982; Lundgren, 1983; Andrews & Lawes, 1984). In this study, we investigated the importance of vagal input in maintaining gastric tone during basal conditions in the dog.

The vagus is a mixed nerve, incorporating cholinergic, adrenergic, and non-adrenergic, non-cholinergic fibres (Roman & Gonella, 1981). Efferent fibres join the nerve trunk at different levels. Cholinergic fibres join the origin of the nerve, whereas adrenergic fibres join the nerve mainly at the thoracic level (Ahlman, Larson, Bombeck & Nyhus, 1979). However, we could not exclude beforehand the possibility of gastric vagal fibres leaving the nerve to join the intramural plexus at the mediastinal oesophagus (as suggested for vagal fibres innervating the lower oesophageal sphincter; Cohen, Kravitz & Snape, 1978; Hall, El-Sharkawy & Diamant, 1982).

Therefore, in studying basal vagal input on gastric tone, we examined the effect of reversible vagal blockade by cooling (Phillipson, Fishman, Hickey & Nadel, 1973; Hall *et al.* 1982; Gleysteen, Sarna & Myrvik, 1985) at either the cervical or the supradiaphragmatic level in conscious dogs. Furthermore, to investigate the nature of vagal fibres modulating gastric tone, we combined the technique of reversible nerve blockade with the administration of either agonist or antagonist autonomic drugs.

METHODS

Gastric barostat

The barostat consists of a strain gauge linked by an electronic relay to an air-injection system. An ultra-thin polyethylene bag (700 ml capacity) is connected to the strain gauge and the injection system of the barostat by a double-lumen 14-F polyvinyl tube. The barostat maintains a constant pressure of 2 mmHg in the air-filled bag positioned within the stomach. When the stomach relaxes,

the system injects air to maintain a constant pressure within the bag; when the stomach contracts, the system aspirates air. Thus, the barostat measures contraction or relaxation of the stomach by recording changes in the volume of air within the intragastric bag maintained at a constant pressure. This approach allows quantitative measurements of variations in gastric tone, as we have validated earlier (Azpiroz & Malagelada, 1985*a*).

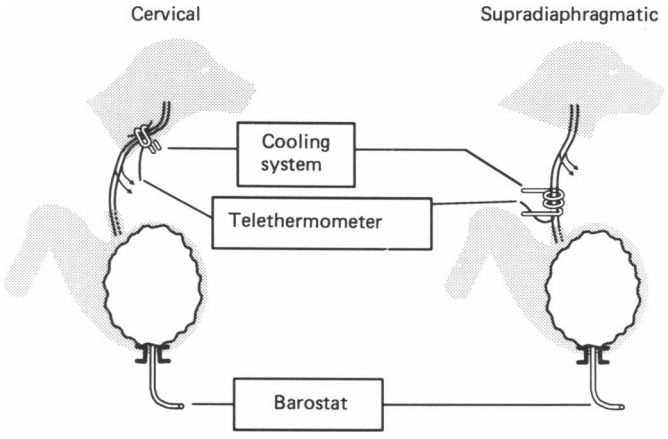

Fig. 1. Canine experimental model: gastric bag of barostat introduced through gastric cannula. Left, cervical vagal isolation within a skin tunnel; external cooling jacket with temperature probe hooked on skin tunnel. Right, supradiaphragmatic vagal isolation within an implanted cooling jacket with temperature probe.

Experimental model

Two different experimental models with chronic vagal isolation were prepared.

Cervical vagal isolation (Fig. 1 left). Three dogs (13–15 kg body weight) were operated on using a sterile technique and general pentobarbitone (pentobarbitone sodium solution, Fort Dodge Laboratories, Fort Dodge, IA, U.S.A.) anaesthesia (26 mg/kg intravenous) and assisted respiration at 4 l/min (respiration pump model 607, Harvard Apparatus, Millis, MA, U.S.A.). Adequate level of anaesthesia was maintained throughout the surgical procedure by the additional administration of pentobarbitone (4 mg/kg intravenous), if required. Through a mid-line cervical incision, the cervical vagi were carefully freed from the carotid sheath and mobilized to the anterior mid line. Both vagal trunks were isolated in a mid-line longitudinal cutaneous tunnel, constructed as follows. A second skin incision, 3 cm long and parallel to the previous cervicotomy, was performed 2·5 cm laterally to the first one. The cutaneous bridge between both incisions was mobilized and wrapped around both vagal trunks by inverting the cutaneous edges. Both edges of the cutaneous strip were then sutured together, thus closing a cutaneous tunnel containing the vagi. The lateral edges of the two skin incisions were sutured below the cutaneous tunnel, closing the cervicotomy. The dogs underwent laparotomy, and a modified gastric Thomas cannula (Thomas, 1941) was implanted in the anterior gastric wall at the level of the distal body and exteriorized in the anterior aspect of the abdomen.

Supradiaphragmatic vagal isolation (Fig. 1 right). In another three dogs (12–15 kg body weight), a right thoracotomy was performed through the seventh intercostal space. The supradiaphragmatic vagi were dissected from the distal oesophagus and mobilized posteriorly. The isolated vagi were then included within a spiral cooling jacket located posterior to the oesophagus, 1 cm cranial to the oesophagogastric junction. The cooling jacket consisted of a stainless-steel tube (2·4 mm outside diameter, o.d., 1·8 mm internal diameter, i.d.) twisted to form a spiral 15 mm long and having an internal coil diameter of 5 mm. A temperature probe (YSI Model 427, Yellow Springs Instruments Co., Yellow Springs, OH, U.S.A.) was introduced within the spiral jacket, with the sensitive surface in contact with the nerves. Both ends of the tubular spiral were connected to plastic tubes that were exteriorized through separate incisions. The cooling jacket was covered with a strip of silicon

rubber (3 mm thick) to provide thermic isolation. The connexion of the temperature probe was also exteriorized, and the thoracotomy was closed. A gastric cannula was implanted through a mid-line laparotomy, as previously described.

Procedure

The dogs were allowed at least 10 days to recover from the operation. Afterward, the dogs were studied under basal conditions, that is, conscious, fasted and resting on a sling. The dogs were observed throughout the experiments, and any sign of discomfort or anomalous behaviour was noted. The bag of the barostat system was introduced through the gastric cannula into the stomach under fluoroscopic control (Machlett Dynascope, Machlett Laboratories Inc., Stamford, CT, U.S.A.) and connected to the barostat (Fig. 1). Using surface electrodes (Silver Stress Test, NDM Corporation, Dayton, OH, U.S.A.), the electrocardiogram (E.C.G.) was monitored in a Hewlett-Packard e.c.g. monitor (model 7830A, Hewlett-Packard Co., Waltham, MA, U.S.A.) provided with a rate computer for continuous recording of the heart rate. An intravenous line was established, and saline was continuously perfused at 1·1 ml/min using a Harvard pump (model 975, Harvard Apparatus Co., Dover, MA, U.S.A.).

Vagal cooling

Vagal cooling was achieved by circulating a cold liquid solution through a cooling jacket positioned around the vagi. (1) For cervical vagal cooling, we used an external cooling jacket consisting of a stainless-steel tube (2·8 mm o.d., 2·2 mm i.d.) bent 180 deg to form a 'U' shape with the arms parallel and 2·5 mm apart. The 'U' tube was bent again 180 deg on itself to form a hook. In each experiment, the cooling jacket was hooked around the cutaneous tunnel containing the vagi. The jacket incorporated a temperature probe (YSI model 427) with its sensitive surface in contact with the skin of the tunnel. (2) For supradiaphragmatic vagal cooling, we used a surgically implanted cooling jacket and temperature probe (see Experimental model). In each experiment, the jacket was connected by the exteriorized tubes to the cooling system.

The temperature probe (either external or surgically implanted) was connected to a telethermometer (model 43TD, Yellow Springs Instruments Co., Yellow Springs, OH, U.S.A.) (Fig. 1). Therefore, vagal temperature was measured either at the surface of the surrounding skin tunnel in the cervical model or at the surface of the nerves in the supradiaphragmatic model.

Vagal temperature was maintained at each predetermined level by circulating, through the cooling jacket, a liquid solution maintained at constant temperature in a reservoir. To reduce the magnitude of the temperature change and consequently to obtain a quicker cooling effect, we maintained during the experiments a base-line or 'control' temperature of 10 °C in the cervical model and a temperature of 15 °C in the supradiaphragmatic model. To produce vagal blockade by cooling, we maintained a temperature of 0 °C in the cervical model and of 5 °C in the supradiaphragmatic model, using a water–alcohol solution kept at freezing point with dry ice. To produce rapid changes in vagal temperature, the liquid reservoir of the cooling system was substituted.

Each single experiment was preceded by a test of vagal cooling to check the permeability of the vagus and the effectiveness of vagal blockade by cooling. In each experiment, vagal cooling was performed four consecutive times. Cooling was maintained for periods of 5 min separated by 5 min periods of vagal rewarming. Vagal temperature (from the telethermometer), intragastric air volume (from the barostat), and heart rate (from the e.c.g. monitor) were transmitted as analog electric impulses to an MFE paper polygraph (model 1600, MFE Corp., Salem, NH, U.S.A.) to obtain permanent tracing (Fig. 1).

Validation studies

These were performed to validate the conductivity of the isolated vagi and the reversible blocking effect of vagal cooling. (1) To determine the actual temperature achieved on the surface of the vagi in the cervical model, we prepared, in two dogs with intact vagi, a chronic cervical skin tunnel (sham tunnel) with an implanted temperature probe (YSI model 427). We studied the effects of cooling the sham tunnel while correlating external and internal temperatures. (2) In two dogs (one of each model), we studied the effect of reducing the vagal temperature from the spontaneous level (body temperature) to the base-line level chosen for the experiments following the procedure described above. (3) To demonstrate vagal conductivity at base-line temperature as well as vagal blockade

at cooling temperature, we studied the effect of vagal cooling on the vagally mediated stimulation of antral motility induced by insulin hypoglycaemia (Bachrach, 1953; Kelly & Code, 1969). In two dogs (one of each model), hypoglycaemia (demonstrated by serial blood glucose determinations) was produced by intravenous bolus injection of 1·5 u/kg insulin (regular iletin, Ely Lilly and Co., Indianapolis, IN, U.S.A.). Antral pressure activity was continuously monitored by two perfused, open-tip manometric catheters (Azpiroz & Malagelada, 1984) introduced into the antrum through the gastric cannula. (4) To prove the completeness of vagal blockade by cooling for 5 min., we performed additional experiments in two dogs, during which we maintained vagal cooling for 60 min. (5) To further prove the completeness of vagal blockade by cooling, we compared the effect on gastric tone produced by vagal cooling with the effect of surgical vagotomy. In two conscious dogs, 1 and 4 days after surgical supradiaphragmatic vagotomy, gastric tone was continuously recorded for 60 min before and 20 min after atropine (atropine sulphate, Elkins-Sinn, Inc., Cherry Hill, NJ, U.S.A.) administration (0·1 mg/kg intravenous bolus). (6) In two dogs, we studied acutely the blocking effect of vagal cooling on bradycardia induced by electric nerve stimulation. Under general pentobarbitone anaesthesia, the cervical vagi were isolated in a skin tunnel and cranially transected. Using a Grass stimulator (SD-9, Grass Instruments Co., Quincy, MA, U.S.A.) and bipolar silver electrodes, we stimulated the peripheral end of the left vagal trunk in quadruplicate (10 Hz, 10 V, 5 ms in 10 s epochs). (7) To verify the absence of nerve damage after completing the reversible cooling studies, we resected for histopathologic examination the cervical skin tunnel containing the vagal trunks (two dogs) or the segment of vagi included within the implanted supradiaphragmatic cooling jacket (two dogs).

Experimental design

We performed experiments of vagal cooling during the continuous intravenous administration of pharmacological agents dissolved in saline (1·1 ml/min infusion rate). Vagal cooling started at least 20 min after beginning the drug infusions in order to allow the heart rate and gastric tone to stabilize. Two sets of experiments, main and ancillary, were performed.

Main experiments. These were performed on each of six dogs in whom the following solutions were infused in randomized order. (1) Placebo: isotonic saline alone. (2) Adrenergic agonist: adrenaline hydrochloride (Elkins-Sinn Inc., Cherry Hill, NJ, U.S.A.), 100 μg/(kg h) infusion. (3) Adrenergic (α and β) blockers: phentolamine mesylate (Regitine, CIBA Pharmaceutical Co., Summit, NJ, U.S.A.), 0·3 mg/kg bolus and 1·5 mg/(kg h) infusion combined with propranolol hydrochloride (Inderal, Ayerst Laboratories, New York, NY, U.S.A.), 0·3 mg/kg bolus and 0·3 mg/(kg h) infusion. (4) Cholinergic agonist: bethanechol chloride (Urecholine, Merck Sharp and Dohme, West Point, PA, U.S.A.), 0·2 mg/(kg h) influsion. (5) Combined adrenergic and cholinergic muscarinic blockers: phentolamine, propranolol (same doses as above), and atropine sulphate (Elkins-Sinn, Inc., Cherry Hill, NJ, U.S.A.), 0·1 mg/kg bolus and 0·1 mg/(kg h) infusion.

Ancillary experiments. To study specific pharmacological aspects we performed ancillary experiments in one dog of each model, following otherwise the same procedure as in the main experiments. (1) To verify that the effect on gastric tone of atropine alone was similar to that of atropine in combination with adrenergic blocking agents (main experiments), we performed an experiment infusing atropine alone. (2) To verify the effect of the doses of blocking agents used in the main experiments, we performed two additional experiments: one, combining the adrenergic agonist (adrenaline) and adrenergic blocking agents (phentolamine and propranolol) and the other, combining the cholinergic agonist (bethanechol) with the cholinergic blocker (atropine). Doses were those used in the main experiments.

Data analysis

We averaged the air volume within the intragastric bag and the heart rate during the 2 min period preceding vagal cooling (base-line level) and during the last 2 min of the cooling period (cooling level). The difference between the two values (before and during cooling) was calculated. For each experiment in each dog, we calculated the mean values of the quadruplicate cooling tests. Grand means and standard errors ($\pm$S.E. of mean) for the group of dogs with a cervical model ($n = 3$) and for the group of dogs with a supradiaphragmatic model ($n = 3$) were then calculated from the individual mean values obtained for each dog. Statistical comparisons between different experiments in each group of dogs ($n = 3$) were performed using Student's t test (two-tailed). Data management of statistical analysis was performed in the CLINFO Data Management System.

RESULTS

Effects of cervical or supradiaphragmatic vagal cooling

Cervical vagal cooling induced a pronounced gastric relaxation, increased the heart rate (Fig. 2 and Table 1), and produced a bilateral Horner's syndrome with evident elevation of the nictitating membrane. These effects disappeared after vagal rewarming. Supradiaphragmatic vagal cooling also produced a reversible gastric relaxa-

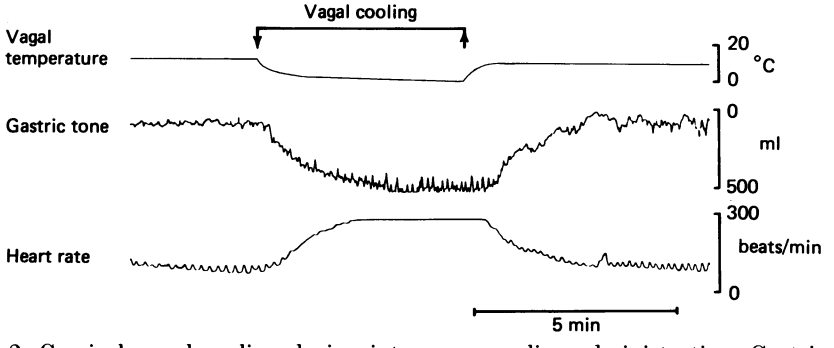

Fig. 2. Cervical vagal cooling during intravenous saline administration. Gastric tone measured by barostat as intragastric volume. Note reversible gastric relaxation (large intragastric volume) and increased heart rate during cooling.

tion, but without the cardiac and ocular effects (Fig. 3 and Table 2). The effects of cervical and supradiaphragmatic vagal cooling on gastric tone were equivalent; therefore, changes in gastric tone in the cervical model were not secondary to the cardio-circulatory effects of vagal cooling. The dogs did not show any sign of discomfort, pain, or anomalous behaviour during the experiments.

Effect of vagal blockade during intravenous infusion of adrenergic agonist or blockers (Tables 1 and 2)

During adrenaline infusion, base-line gastric tone was significantly lower than during saline infusion. However, vagal cooling at either a cervical or a supradiaphragmatic level still produced a significant gastric relaxation (Fig. 4). Adrenaline infusion augmented the increase in heart rate produced by cervical cooling. Throughout these experiments, adrenaline induced cardiac extrasystoles with frequent episodes of bigeminous rhythm.

Adrenergic blocking agents did not significantly change base-line gastric tone, heart rate, or cooling-induced gastric relaxation (Fig. 5). However, heart rate was significantly lower during cervical vagal cooling than during saline infusion.

Ancillary experiments combining an adrenergic agonist with an adrenergic blocking agent showed that base-line and cooling levels of gastric tone as well as heart rate were similar to those obtained with adrenergic blocking agents alone in the same dogs (data not shown). Therefore, the dose of the adrenergic antagonist used in the main experiments completely blocked an effective dose of an adrenergic agonist.

Effect of vagal blockade during intravenous infusion of cholinergic agonist or blocker (Tables 1 and 2)

Cholinergic stimulation increased base-line gastric tone (not statistically significant) and completely suppressed the gastric relaxation induced by cooling (Fig. 6). Bethanechol infusion blunted the increase in heart rate produced by cervical cooling. Bethanechol induced profuse salivation in all the experiments.

TABLE 1. Effect* of cervical vagal cooling on gastric tone and heart rate

Intravenous infusion	Gastric tone (intragastric volume, ml)			Heart rate (beats/min)		
	Base line	Cooling	Δ cooling	Base line	Cooling	Δ cooling
Saline	98±39	320±54	221±17†	111±8	216±8	106±1†
Adrenergic agonist	354±53‡	419±56	65±11†	111±14	261±15	150±29†
Adrenergic blockers	188±43	418±33	231±37†	105±17	161±17‡	56±2†
Cholinergic agonist	49±12	52±15‡	0±4	102±6	187±9	84±15†
Cholinergic and adrenergic blockers	423±14‡	423±10	−1±4	162±16‡	166±16‡	4±1

* Mean±S.E. of mean ($n = 3$ dogs).
† Significant at $P < 0.05$.
‡ Significant difference from respective saline value ($P < 0.05$).

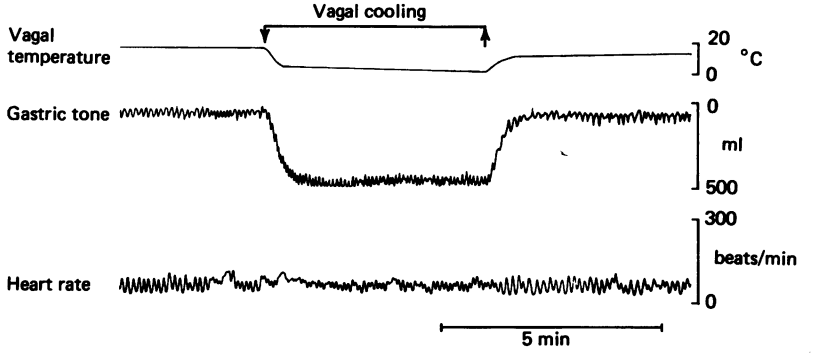

Fig. 3. Supradiaphragmatic vagal cooling during intravenous saline administration. Note pronounced gastric relaxation without change in heart rate.

During combined infusion of atropine and adrenergic blocking agents, base-line gastric tone was low and heart rate was increased; neither were they modified by vagal cooling.

Ancillary experiments in which atropine was infused alone (Fig. 7) showed that the levels of gastric tone (457 ml base-line volume; mean for two dogs) were equivalent to those observed when atropine and adrenergic blocking agents were combined. However, base-line heart rate in both dogs was higher with atropine alone (242 beats/min; mean for two dogs) than when atropine and adrenergic blocking agents were combined. Vagal cooling changed neither gastric tone nor heart rate.

Ancillary experiments in which bethanechol and atropine were combined showed that the levels of gastric tone and heart rate were similar to those obtained with

TABLE 2. Effect* of supradiaphragmatic vagal cooling on gastric tone and heart rate

Intravenous infusion	Gastric tone (intragastric volume, ml)			Heart rate (beats/min)		
	Base line	Cooling	Δ cooling	Base line	Cooling	Δ cooling
Saline	103±21	376±81	275±91†	92±16	95±17	3±1
Adrenergic agonist	291±7‡	391±20	101±15†	134±18	129±12	−5±6
Adrenergic blockers	64±8	351±88	287±87†	115±13	113±11	−2±2
Cholinergic agonist	47±32	46±31‡	−1±1	127±10	130±8	3±1
Cholineric and adrenergic blockers	400±50‡	408±49	9±2	186±5‡	188±6‡	2±1

* Mean±S.E. of mean ($n = 3$ dogs).
† Significant at $P < 0{\cdot}05$.
‡ Significant difference from respective saline value ($P < 0{\cdot}05$).

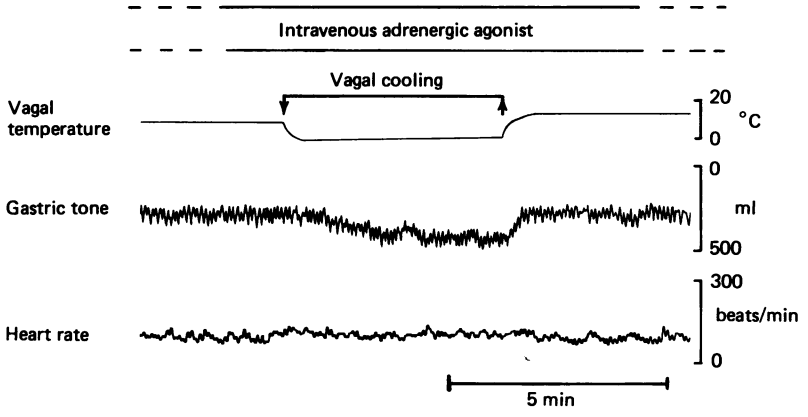

Fig. 4. Supradiaphragmatic vagal cooling during intravenous adrenaline administration. Note low base-line gastric tone (large intragastric volume) and further relaxation during cooling.

atropine alone in the same dogs (data not shown). Therefore, the dose of atropine used in the main experiments blocked an effective dose of the cholinergic agonist.

Validation of the vagal cooling technique

(1) Cooling of the cervical sham tunnel did not change gastric tone (−4 ml Δvol; mean for two dogs) or heart rate (−5 beats/min Δ heart rate; mean for two dogs). Therefore, the effects observed in the cervical model were not due to cutaneous cooling. Internal temperature within the cervical sham tunnel (measured by an implanted temperature probe) was approximately 5 °C higher than the external (skin) temperature for both 'base-line' and 'cooling' temperatures (4·8 °C temperature difference; mean for two dogs). Therefore, actual temperature on the surface of the vagi in the cervical model was the same as in the supradiaphragmatic model (15 °C base line and 5 °C cooling). Changes in temperature was transmitted inside the skin tunnel with a lag time of 33 s (lag time measured for the 90% of the maximal

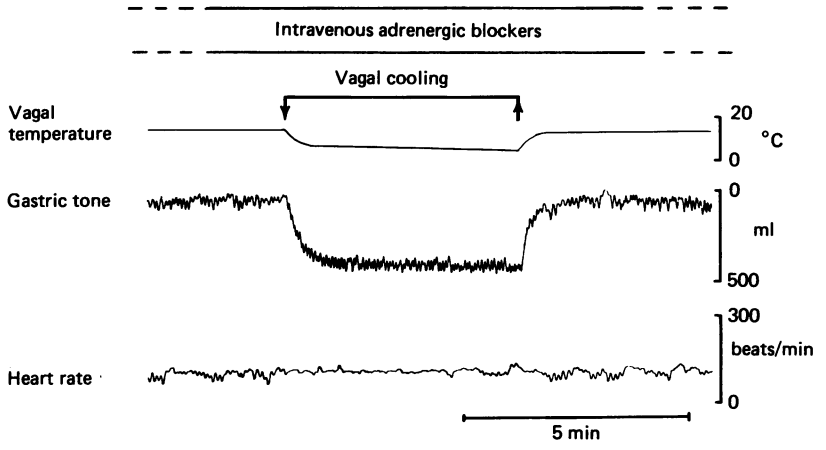

Fig. 5. Supradiaphragmatic vagal cooling during intravenous administration of phentolamine plus propranolol. Adrenergic blocking agents did not affect base-line gastric tone nor cooling-relaxation.

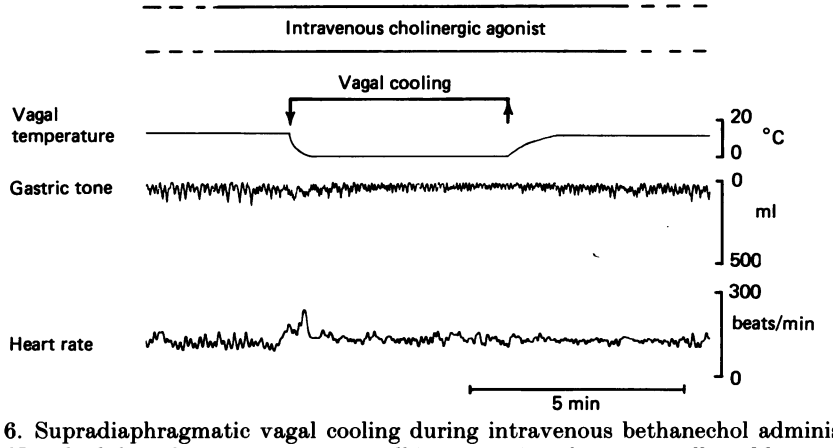

Fig. 6. Supradiaphragmatic vagal cooling during intravenous bethanechol administration. Note high base-line gastric tone (small intragastric volume) not affected by cooling.

temperature change; mean for two dogs). (2) Spontaneous temperature in the cervical model was 29·9 ± 0·7 °C (measured on the skin of the cervical tunnel) and 39·3 ± 0·4 °C in the supradiaphragmatic model (temperature probe in contact with the vagi). Reducing spontaneous vagal temperature to the base-line level chosen for the studies did not change gastric tone or heart rate in either the cervical model or the supradiaphragmatic model (data not shown). (3) Insulin administration reduced blood glucose concentration by > 45% and greatly increased antral contractility (pressure waves > 50 mmHg at a rate of > 3/min) throughout the experimental period (20–80 min after insulin injection). In both the cervical and the supradiaphragmatic models, antral pressure activity was completely abolished by vagal cooling and reappeared after rewarming the vagi to base-line temperature (Fig. 8). (4) Gastric tone and heart rate averaged over 2 min periods after 10, 30 and 50 min of

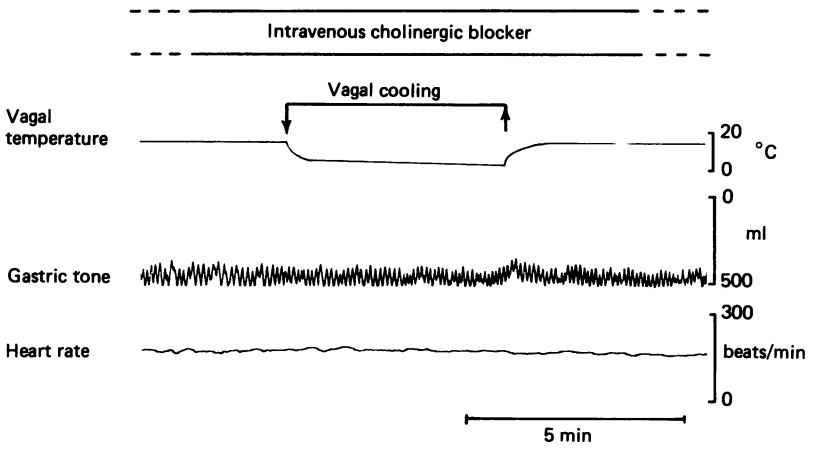

Fig. 7. Supradiaphragmatic vagal cooling during intravenous atropine administration. Note very low base-line gastric tone (large intragastric volume) and no change during cooling.

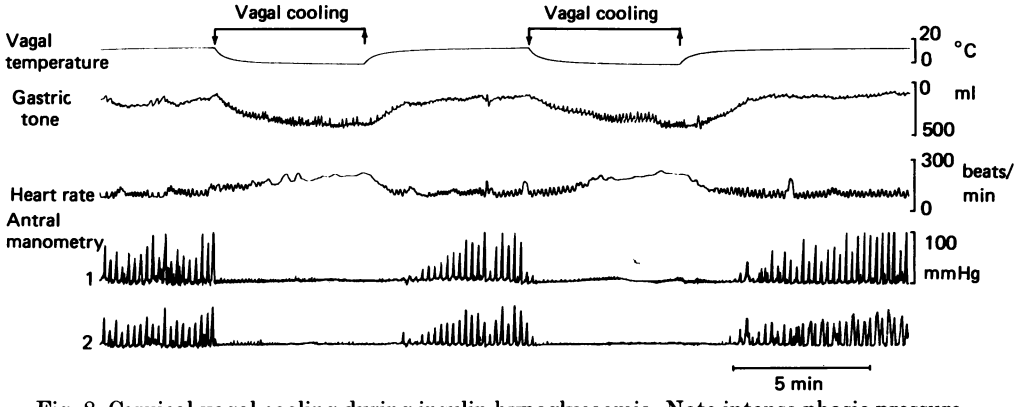

Fig. 8. Cervical vagal cooling during insulin hypoglycaemia. Note intense phasic pressure activity in the antrum suppressed by vagal cooling. Two different sites of manometry in the antrum are indicated in the two bottom traces (1 and 2).

continuous vagal cooling were similar to the levels achieved after 3 min cooling in the same dogs (data not shown). (5) Gastric tone studied 1 day (350 ml; mean for two dogs) and 4 days (321 ml; mean for two dogs) after surgical supradiaphragmatic vagotomy was similar to the level achieved during vagal cooling in previous experiments in the same dogs. After surgical vagotomy, atropine had no significant effect on gastric tone (12 ml Δ vol; mean for two dogs). (6) After cervical vagal transection under general anaesthesia, heart rate was increased (195 beats/min; mean for two dogs). Electrical stimulation of the peripheral end of the left vagus produced a large decrease in heart rate (−70 beats/min Δ heart rate; mean for two dogs). Vagal cooling distal to the stimulating electrodes reversibly blocked this effect. (7) Pathological examination of serial sections of cervical or supradiaphragmatic vagal nerve specimens after completion of the experiments showed a normal nerve structure surrounded by connective tissue in all instances.

DISCUSSION

Our results provide for the first time a conclusive experimental basis to support the concept of a vagal cholinergic excitatory input on canine gastric tone under basal conditions (conscious, fasting and resting animals).

We have demonstrated that vagal blockade produces gastric relaxation, which indicates a vagal (extrinsic) excitatory input on gastric tone. *In vivo* studies that record the spontaneous activity of vagal efferent fibres during basal conditions in the dog also suggest the existence of a tonic excitatory input to the stomach (Roman & Gonella, 1981; Miolan & Roman, 1984). Because cholinergic blockade (atropine) mimics the effect of vagal blockade and because a cholinergic agonist (bethanechol) increases gastric tone regardless of vagal blockade, the excitatory vagal input probably is mediated by a cholinergic (muscarinic) mechanism. This conclusion is supported by *in vitro* investigations that show a gradual depolarization and consequent tonic contraction of fundic muscle fibres in response to neural cholinergic stimulation (Morgan *et al.* 1981). Consequently, our data indicate that basal gastric tone is maintained by an extrinsic cholinergic input, which is vagally mediated at both the cervical and the supradiaphragmatic levels.

The observed lack of effect of adrenergic blocking agents on basal gastric tone indicates the absence of basal adrenergic input on gastric tone during fasting. Adrenergic stimulation, either pharmacological, as in our experiments, or physiological, as it occurs during stress (Stanghellini, Malagelada, Zinsmeister, Go & Kao, 1983), may induce gastric relaxation. Gastric relaxation has also been demonstrated in response to electrical stimulation of the greater splanchnic nerve (Andrews & Lawes, 1984). This adrenergically mediated relaxation may involve a dual effect: inhibition of acetylcholine release from intramural cholinergic neurones (α-receptors) and direct inhibition of the smooth muscle (β-receptors) (Roman & Gonella, 1981).

Non-adrenergic, non-cholinergic input may also have a relaxatory effect on gastric tone (Martinson, 1965; Morgan *et al.* 1981). The lack of appropriate agonists and antagonists precluded specific studies on this pathway. However, interruption of this pathway would produce gastric contraction, which is the opposite effect that we have demonstrated with vagal blockade. Thus, during basal conditions, the vagal input on gastric tone seems to be predominantly cholinergic excitatory.

Unexpectedly, vagotomy in previous studies consistently resulted in an increased gastric tone (Harper *et al.* 1959; Martinson, 1965; Jansson, 1969; Martinson, 1975; Jahnberg, 1977; Andrews & Lawes, 1982; Lundgren, 1983; Andrews & Lawes, 1984), except for a single report of transient decrease in gastric tone (lasting about 10 min) after vagotomy (Andrews & Scratcherd, 1980). These results led to the conflicting conclusion that 'vagotomy causes an increase in gastric tone, quite opposite to the old view considering vagotomy to cause atony of the stomach' (Martinson, 1975). A careful review of the published work reveals that previous studies were performed under conditions other than basal (that is, gastric distension, acute experimental conditions, chronic vagotomy). This may account for the incongruent results. Gastric distension disrupts the interdigestive (basal) motor pattern in the dog (Azpiroz & Malagelada, 1984) and may induce gastric relaxatory reflexes (Jahnberg, 1977; Andrews, Grundy & Lawes, 1980). Similarly, surgical manoeuvres or anaesthesia in acute experiments may also disrupt the interdigestive motor pattern (Smith *et al.*

1977; Diamant *et al.* 1985) and induce changes in gastric tone (Martinson, 1965; Glise & Abrahamson, 1984). Chronic vagotomy, however, may be followed by adaptative changes secondary either to the lack of trophic effect consecutive to denervation or to the release of mechanisms due to the suppression of feed-back control (Hall *et al.* 1982; Håkanson, Vallgren, Ekelund, Rehfeld & Sundler, 1984).

A unique methodological feature of our model is that it allows prolonged study of gastric tone and its control under basal conditions. During the experiments, the fasting dogs were quiet, resting on a sling without signs of discomfort or stress. As previously validated, the gastric barostat does not distort the interdigestive motor pattern, probably because the low and constant intragastric pressure (muscular wall tension) does not stimulate tension receptor on the gastric wall (Azpiroz & Malagelada, 1985*a*). Vagal cooling achieves reversible nerve blockade in conscious animals without interrupting the interdigestive intestinal motor pattern (Hall *et al.* 1982; Gleysteen *et al.* 1985). *In vitro* studies have demonstrated blocked conduction in vagal fibres at variable temperatures (mean, 7·5 °C), depending on the type of fibres, nerve tension, external pressure and length of the cooled segment (Douglas & Malcolm, 1955; Paintal, 1965).

During the experiments, we maintained a base-line vagal temperature of 15 °C. We demonstrated that the conductivity of the vagus at this temperature was preserved by showing, first, that heart rate and gastric tone did not change when vagal temperature was reduced from the spontaneous level (body temperature) to 15 °C and secondly, that the excitatory effect of insulin hypoglycaemia on antral motility was unaffected by cooling to 15 °C. This effect of insulin hypoglycaemia requires vagal innervation (Bachrach, 1953; Kelly & Code, 1969). Additional steps were taken to verify that vagal conductivity was preserved after repeated cooling and rewarming throughout the experiments by pre-testing the effects of cooling on each experimental day. Furthermore, the nerves showed no microscopic damage after completion of the experiments.

To achieve vagal blockade, we reduced vagal temperature to 5 °C. We were concerned because of the impossibility of demonstrating a complete blockade of all vagal fibres by cooling. For instance, we cannot exclude the possibility that the effects observed here are secondary to cooling of afferent fibres that reflexly drive a population of efferent fibres that are not blocked at 5 °C. However, every validation experiment on the blocking effect of vagal cooling was consistent with complete blockade at 5 °C. First, tachycardia was induced by cooling cardiac fibres in the cervical vagus. Secondly, bradycardia induced by electrical stimulation of the transected vagi in the neck was suppressed. Thirdly, ocular fibres were blocked in the cervical model (Horner's syndrome; Phillipson *et al.* 1973; Hall *et al.* 1982). Fourthly, antral motility induced by insulin hypoglycaemia was abolished. Fifthly, prolonged cooling (1 h) or surgical vagotomy resulted in the same effects as the 5 min cooling to 5 °C used to induce reversible blockade.

In conclusion, our results indicate that gastric tone in the fasted state is maintained by a cholinergic vagal input, and although there is no basal adrenergic input on gastric tone, adrenergic stimulation may produce gastric relaxation. These findings expand our understanding of the physiological control of gastric tone and may help interpret the pathophysiology of post-surgical or other neuropathic disorders of gastric motility.

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