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EFFECTS OF SUBDIAPHRAGMATIC VAGOTOMY ON ENERGY BALANCE AND THERMOGENESIS IN THE RAT

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SUMMARY

1. Subdiaphragmatic vagotomy caused chronic gastric distension and hypertrophy, and a reduction in voluntary food intake in rats fed a pelleted stock diet. These effects were minimized by feeding a more digestible semisynthetic diet.

2. Vagotomized rats fed the pelleted diet showed lower rates of oxygen consumption than pair-fed controls, and the rise in metabolic rate (thermic response) following gastric intubation with a carbohydrate meal was diminished. This could be restored to normal by simultaneous injection of insulin. Thermic responses to fat and noradrenaline were normal in the vagotomized group.

3. On the powdered semisynthetic diet, vagotomized rats gained more weight and showed greater efficiency of energy gain than pair-fed controls. The thermic response to a single meal of the semisynthetic diet was depressed in these vagotomized rats, but restored to normal by acute insulin treatment.

4. The activity of the thermogenic proton conductance pathway in brown adipose tissue mitochondria (assessed from purine nucleotide binding) was reduced by vagotomy in animals on both diets, but was restored to normal by chronic insulin treatment, which also slightly raised brown fat activity in sham-operated rats.

5. These results demonstrate that the reduced gastric activity and food intake following vagotomy is dependent on the digestibility and/or composition of the diet. When differences in food intake are abolished by pair feeding, vagotomy reduces thermogenic responses to carbohydrate, probably as a result of impaired insulin release. This may be responsible for the enhanced energetic efficiency and elevated weight and energy gains seen after vagotomy.

INTRODUCTION

Variations in energy intake in laboratory rodents often produce compensatory changes in energy expenditure, known as diet-induced thermogenesis, which tend to stabilize body weight and energy balance (see Rothwell & Stock, 1981, 1983 for reviews). Chronic food restriction causes a fall in metabolic rate in laboratory rodents (Rothwell & Stock, 1982*a*), whereas hyperphagia can induce increases in energy expenditure of up to 80% (Rothwell & Stock, 1982*b*,*c*). These adaptive changes in heat production appear to result largely from sympathetic activation of thermogenesis

in brown adipose tissue (b.a.t.) via a proton conductance pathway which causes uncoupling of oxidative phosphorylation (see Landsberg & Young, 1983; Rothwell & Stock, 1984; Nicholls & Locke, 1984, for reviews). In addition to chronic changes in food intake, acute ingestion of a single meal can also raise heat production (the thermic effect of food), and this appears also to be partly due to sympathetic activation of b.a.t. (Rothwell, Saville & Stock, 1981).

Defective diet-induced thermogenesis, resulting from decreased sympathetic nervous system or b.a.t. activity may be fundamental to the development of obesity in genetically obese rats and mice and those which have suffered destruction of the ventromedial hypothalamus (Jeanrenaud, 1978; Bray & York, 1979; Trayhurn & James, 1983). Hyperinsulinaemia has been suggested as a major cause of hyperphagia and associated metabolic defects in obese animals (Powley, 1977; Jeanrenaud, 1978) and is thought to be due to increased vagal efferent activity, since subdiaphragmatic vagotomy, transplantation of pancreatic β cells, or experimentally induced diabetes all attenuate or prevent the obesity induced by ventromedial hypothalamic damage (York & Bray, 1972; Powley & Opsahl, 1974; Inoue & Bray, 1977; Inoue, Bray & Mullen, 1978). However, several workers have failed to prevent excess fat deposition by vagotomy (King, Carpenter, Stamoutsos, Frohman & Grossman, 1978; Wampler & Snowdon, 1979; King, Phelps & Frohman, 1980).

Vagotomy usually results in impaired gastric motility and gastric distension (Powley & Opsahl, 1974; Rowland & Engle, 1978), which will tend to modify food intake and hence alter circulating insulin levels. Many studies on the effects of vagotomy have maintained animals on conventional, pelleted stock diets, which contain large quantities of indigestible carbohydrate and will therefore exacerbate these problems. The effects of vagotomy on energy balance, thermogenesis or b.a.t. function have not been investigated, although Cox & Powley (1981) did conclude from a pair-feeding study, that this treatment may normalize the metabolic defects resulting from ventromedial hypothalamic lesions. In the present study we have investigated the effects of subdiaphragmatic vagotomy on these parameters and have also compared the influence of this treatment on animals consuming diets of different digestibilities, and during chronic treatment with insulin.

METHODS

All animals used in this study were male, Sprague–Dawley rats aged 55 days (Charles River, Kent), which were housed in pairs in a metabolism room at 24 ± 1 °C (light phase 08.00–20.00 h).

Subdiaphragmatic vagotomy or sham operations were performed under halothane anaesthesia (2% halothane in oxygen/nitrous oxide mixture). The fur over the abdomen was shaved and an incision (6 cm) was made, starting just below the xiphisternum. The liver and intestines were gently displaced to provide access to the lower oesophagus and stomach, and the dorsal and ventral abdominal vagal trunks were identified running close to the oesophagus. The trunks were mobilized over a distance of approximately 1 cm, ligated, and a segment of nerve at least 5 mm long removed from between the ligatures. If an hepatic branch was observed leaving the ventral vagal trunk, the trunk was ligated and sectioned above this division. In all the above animals the gastro-oesophageal junction region was stripped of any strands of tissue that might have contained vagal fibres.

In sham-operated rats, the liver and gastrointestinal tract were manipulated but the vagi were not sectioned. In both groups the incision was closed by suturing and all animals recovered

consciousness within 20 min post-operatively. No deaths resulted from vagotomy, and all rats were seen to be eating small amounts of food within 2–3 h after recovery from anaesthesia.

Experiment 1

In this preliminary experiment, five sham-operated and six vagotomized rats were maintained on a standard pelleted stock diet (pig rearing diet, PRD, Christopher Hill Group Ltd., Dorset; for details of composition, see Table 1) for 21 days after surgery. Sham-operated rats were presented with the same weight of food as vagotomized animals had consumed on the previous day in order to achieve similar intakes. Spilt food was weighed each day and metabolizable energy intakes calculated from the weights of food consumed and the metabolizable energy density of the diet (12 kJ/g, determined in previous experiments in this laboratory).

Resting oxygen consumption ($\dot{V}_{O_2}$) was measured on four occasions (days 11, 14, 17, 19) in closed-circuit respirometers (Stock, 1975) at 29 °C for 2 h, or until steady base-line values had been achieved. $\dot{V}_{O_2}$ was then measured for a further 2–3 h following either a single injection of noradrenaline (25 μg/100 g body weight, s.c.), gastric intubation with carbohydrate (40 kJ, 2·5 g cornflour in 4 ml water), fat (40 kJ, 0·8 ml corn oil) or carbohydrate plus insulin (1 u. Actrapid, Novo, Basingstoke) injected subcutaneously at the start of the resting period and again at the time of intubation. Both groups were allowed free access to food until 08.00 h on the day when the response to noradrenaline was measured. Food was removed from all animals at 19.00 h on the night prior to measurements of $\dot{V}_{O_2}$ in response to intubation of fat or carbohydrate, in order to avoid problems of severe stomach distension, but both groups were given a 20 % glucose solution to drink overnight. The volume of solution consumed was similar for control and vagotomized rats and this treatment has no significant effect on the resting $\dot{V}_{O_2}$ or the thermic response to food when compared to the response in freely-fed animals (N. J. Rothwell & M. J. Stock, unpublished data; and Table 2). All values for $\dot{V}_{O_2}$ were taken when the animals were resting and have been corrected for body size ($ml/kg^{0.75}$. min).

At the end of the experiment (day 21), all rats were anaesthetized (urethane 0·12 mg/100 g), and the interscapular b.a.t. depot was carefully dissected, removed and processed as described below. The wound site was packed with surgical gauze. A cannula was placed in the trachea and blood pressure monitored from the right common carotid artery. The cervical vagi were mobilized, ligated, sectioned and prepared for stimulation of their peripheral cut ends as previously described (Andrews & Scratcherd, 1980). The stomach was exposed via a mid-line abdominal incision and the pylorus ligated. The girth of the stomach was measured at the junction between the forestomach and the antrum. A 5 mm long incision was made along the greater curve in the forestomach and the gastric contents removed by gently massaging the stomach. The contents were collected and the wet and dry weights measured. Particular attention was paid to ensuring that sham and vagotomized animals had a similar degree of gastric manipulation. The gastric incision was closed with a ligature taking care to minimize damage to the gastric wall. The stomach was intubated via the mouth and oesophagus, and the cannula secured by a ligature around the cervical oesophagus after ensuring that it was in the stomach. The abdominal incision was closed with surgical clips. The animals' temperatures were monitored from a rectal probe and maintained at 37 °C by a homoeothermic blanket (Palmer Bioscience, Kent). Blood pressure and intra-gastric pressure were both monitored (Palmer 1838 blood pressure monitors) and displayed on chart recorders (Gould 2400, Lutterworth; Bryans 2800, Mitcham). Heart rate was intermittently measured from the blood pressure record. After completion of the surgery, the animal was left for at least 30 min before the vagi were stimulated. At the end of the experiment the animals were killed by an anaesthetic overdose and the stomach removed, dissected into antral and forestomach regions and weighed. The tissue was reweighed after drying to constant weight in an oven at 80 °C.

The interscapular b.a.t. was placed on ice immediately after dissection, minced and homogenized in 0·2 M-sucrose. Mitochondria were prepared (Slinde, Pederson & Flatmark, 1975) and the activity of the proton conductance pathway was assessed from the binding of [^{3}H]guanosine diphosphate (GDP, 10 Ci/mmol, Amersham International, Bucks) to isolated mitochondria. Specific GDP-binding was assessed using a concentration of 2 μM ligand with displacement by excess (200 μM) unlabelled nucleotide to determine non-specific binding (Brooks, Rothwell & Stock, 1982). Mitochondrial protein contents were estimated using a protein dye-reagent assay (Bio-Rad, Watford), with bovine serum albumin standards.

Experiment 2

Six rats (as above) were killed on the first day of the experiment for determination of initial body energy content (Bo group) and a further sixteen weight-matched animals were subjected to subdiaphragmatic vagotomy or sham operations. Both groups ($n = 8$) were maintained on a powdered semisynthetic diet (Complan, Glaxo, see Table 1 for composition) throughout the experiment (20 days), and sham-operated rats were pair-fed to the same level of intake as the vagotomized group.

TABLE 1. Composition of diets used

	Pelleted diet PRD (Expt. 1)	Semisynthetic diet Complan (Expts. 2 and 3)
	(% metabolizable energy)	
Carbohydrate	64	50
Fat	9	32
Protein	28	18
Metabolizable energy density (kJ/g)	12·0	18·1
Digestibility (%)	70	92

Nutrient composition was obtained from manufacturers values. Metabolizable energy density and digestibility were determined in separate feeding trials (Rothwell & Stock, 1982*b* and unpublished data).

Metabolizable energy intake of both groups was assessed from the gross energy density of the diet (determined by ballistic bomb calorimetry) minus the energy lost in any spilt food, urine and faeces.

Resting $\dot{V}_{O_2}$ was measured on day 7 for 2 h before and 3 h after gastric intubation with the semisynthetic diet (40 kJ, 2·25 g Complan in 4 ml water) and on day 10 in response to the same meal after injection of insulin (1 u. Actrapid, S.C.) on day 10. All rats were given glucose solution, but no food overnight before the measurements (as above).

At the end of the experiment (day 20) all rats were anaesthetized and the interscapular b.a.t. was dissected and mitochondria prepared for determination of the activity of the proton conductance pathway (see above). The animals were then prepared for vagal stimulation, monitoring of blood pressure and intra-gastric pressure and measurement of stomach girth and weight as described above.

All carcasses were chopped, freeze-dried, homogenized and energy content determined by ballistic bomb calorimetry. Body energy gain was determined from the final energy content of each rat minus its initial energy content, estimated from the energy density of the initial Bo group. Energy expenditure over the entire experiment was assessed from metabolizable energy intake minus body energy gain, gross energetic efficiency from body energy gain per unit intake, and net energetic efficiency from body energy gain per unit intake above maintenance, using the interspecific value of 420 $kJ/kg^{0.75}$. day for the maintenance requirements of each rat.

Experiment 3

Twelve rats were subjected to subdiaphragmatic vagotomy and a further twelve received sham operations, as in the earlier experiments. All animals were maintained on semisynthetic diet (Complan) and housed as above, but half of each group (vagotomized and sham) were given daily injections of insulin (protamine zinc insulin, 4 u./day) for 20 days. The food intake of sham-operated rats was restricted to that of their respective vagotomized group. At the end of the experiment, the interscapular b.a.t. depot was removed and specific mitochondrial GDP-binding measured as in Expt. 2.

Values are presented as means $\pm$S.E. of means. Significant differences were assessed by the Student's t test for unmatched data using two-tailed probabilities.

RESULTS

(*a*) *Test for vagotomy*

The completeness of the subdiaphragmatic vagotomy in all groups was tested by monitoring the intra-gastric pressure in response to stimulation (20 V, 0·5 ms, 20 s, 10 and 20 Hz) of the peripheral cut ends of the cervical vagi. The vagi were stimulated when the stomach contained 2 ml and 5 ml 154 mM-NaCl. Both sham-operated and subdiaphragmatic vagotomy groups showed a characteristic bradycardia and consequent fall in blood pressure when either cervical vagus was stimulated. During the surgical preparation for vagal stimulation it was noted that when the cervical vagi were sectioned the respiratory rate slowed and the depth increased in both groups of animals.

In the sham-operated animals intra-gastric pressure usually increased during the period of stimulation and fell below the pre-stimulation levels when the stimulus stopped. The pressure took several minutes to return to control levels. In some animals only a decrease in intra-gastric pressure was observed during vagal stimulation.

Animals with a subdiaphragmatic vagotomy did not show any significant change in intra-gastric pressure in response to cervical vagal stimulation. Both left and right vagi were tested for their influence on the stomach and for the vagotomy to be considered complete both had to show a negative response. These experiments indicated that at least as far as the stomach was concerned, the subdiaphragmatic vagotomies were complete and it is likely that the remainder of the abdominal viscera were also vagally denervated. The criteria for vagotomy are considered further in the discussion.

(*b*) *The effects of vagotomy on animals fed a pelleted diet* (*Expt. 1*)

The vagotomized rats fed the pelleted diet (PRD) consumed approximately 30% less energy than intact free-feeding rats of the same age. The reduction in intake in the vagotomized group was probably due to changes in gastrointestinal function associated with vagotomy. The abdomens of these animals appeared swollen, particularly during the early part of the experiment, and their stomachs were markedly distended (girth: sham 37 ± 7 mm, vagotomized 97 ± 10 mm, $P < 0{\cdot}001$) and contained significantly more food (vagotomized $15{\cdot}5 \pm 2{\cdot}4$ g) than controls ($1{\cdot}6 \pm 0{\cdot}9$ g, $P < 0{\cdot}01$) at the end of the experiment. All values for food intake were corrected for the food left in the stomach.

Vagotomized rats showed a normal pattern of feeding, consuming most of their food during the dark phase (20.00–08.00 h), but because of the food restriction, controls ate most of their food during the early part of the day (09.00–14.00 h). Total energy intake (kJ) did not differ significantly between control (4410 ± 60 kJ) and vagotomized rats (3535 ± 330 kJ) maintained on pelleted diet (Expt. 1), however, intake corrected for body size (kJ/kg$^{0{\cdot}75}$.day) was significantly ($P < 0{\cdot}05$) reduced by 17% in vagotomized rats (sham 660 ± 10, vagotomized 550 ± 25). In spite of this, body weight gain over the 21 day period of the experiment was identical for intact (31 ± 4 g) and vagotomized rats (31 ± 16 g), so that feed efficiency (g gain/MJ eaten) was slightly enhanced by vagotomy (control $7{\cdot}0 \pm 0{\cdot}8$ g, vagotomized $8{\cdot}8 \pm 1{\cdot}2$ g).

Resting $\dot{V}_{O_2}$ was significantly reduced in vagotomized rats (8–17 % below controls, Table 2). Injection of noradrenaline provoked a marked increase in $\dot{V}_{O_2}$, which was of similar magnitude (Table 2) and duration (peak 40–60 min) in control and vagotomized rats. Gastric intubation with fat produced an increase in $\dot{V}_{O_2}$ of 20 % (peak oxygen consumption was between 60 and 90 min) in all animals and metabolic rate remained approximately 10 % above preprandial levels at the end of the

TABLE 2. Oxygen consumption and brown fat of control and vagotomized rats fed a pelleted diet

	Control	Vagotomized
$\dot{V}_{O_2}$ (ml/kg$^{0.75}$. min)		
Before noradrenaline	12·59 ± 0·36	10·42 ± 0·53**
After noradrenaline	20·44 ± 0·69	16·31 ± 1·42*
Increase (%)	63·1 ± 6·7	55·3 ± 6·4 N.s.
Before fat	11·92 ± 0·37	10·16 ± 0·37**
After fat	14·31 ± 0·70	12·35 ± 0·53
Increase (%)	19·9 ± 2·3	21·5 ± 1·1 N.s.
Before carbohydrate	12·32 ± 0·24	11·38 ± 0·28*
After carbohydrate	14·52 ± 0·36	12·26 ± 0·26***
Increase (%)	17·9 ± 1·2	7·9 ± 1·4***
Before carbohydrate	11·31 ± 0·44	10·51 ± 0·36 N.s.
After carbohydrate + insulin	13·24 ± 0·60	12·19 ± 0·51 N.s.
Increase (%)	16·9 ± 1·7	15·9 ± 2·2 N.s.
Brown adipose tissue		
Interscapular b.a.t. mass (mg)	270 ± 29	205 ± 35 N.s.
Protein content (mg)	14·6 ± 1·1	11·9 ± 1·1 N.s.
(%)	5·5 ± 0·3	6·1 ± 0·6 N.s.
Mitochondrial protein (mg)	2·50 ± 0·25	1·98 ± 0·27 N.s.
Specific GDP binding (pmol/mg protein)	22 ± 1	15 ± 2***

Data from Expt. 1. The effects of a single injection of noradrenaline or gastric intubation with 40 kJ fat or carbohydrate on oxygen consumption, and interscapular b.a.t. mass and activity from the same animals.

Mean values ± S.E. of mean. *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ compared to controls. N.s., not significantly different.

measurements (2–3 h after feeding). The rise in metabolic rate after fat was very similar for both groups, although the pre- and post-prandial levels of oxygen consumption were lower in vagotomized rats. Intubation with carbohydrate produced an 18 % increase in $\dot{V}_{O_2}$ in sham-operated rats but only an 8 % increase in vagotomized animals (peak values occurring at approximately 80 min after intubation). The increment in $\dot{V}_{O_2}$ after intubation with carbohydrate was reduced by almost 60 % in vagotomized compared to intact rats. Injection of insulin did not significantly affect the response to carbohydrate in control animals, but significantly enhanced the effect in the vagotomized group. Thus, after insulin treatment, vagotomized rats showed a similar carbohydrate-induced increase in $\dot{V}_{O_2}$ to controls, and this was almost twice as large as the response seen in the absence of insulin.

The weight of the forestomach (control 0·43 ± 0·02 g, vagotomized 1·06 ± 0·07 g, $P < 0·001$) and antrum (control 0·85 ± 0·06 g, vagotomized 1·50 ± 0·08 g, $P < 0·05$)

were significantly increased by vagotomy, but the tissue water content of the two gastric regions was not significantly changed by vagotomy. The mass, protein content and mitochondrial protein of the interscapular b.a.t. depot were not significantly affected by vagotomy (Table 2). However, the activity of the mitochondrial proton conductance pathway, assessed from the specific binding of [^{3}H]GDP, was depressed by 32% in vagotomized rats compared to controls.

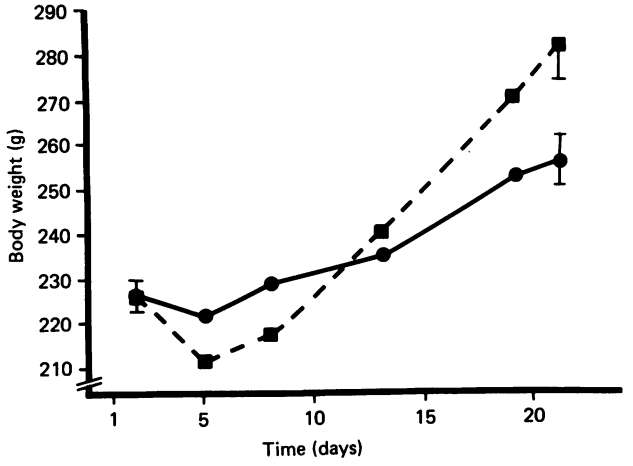

Fig. 1. Changes in body weight of vagotomized (■) rats and pair-fed control rats (●) maintained on the semisynthetic diet (Expt. 2). Mean values, $n = 8$; bar represents S.E. of mean.

(*c*) *The effects of vagotomy on the animals fed the powdered semisynthetic diet* (*Expt. 2*)

Animals fed the powdered semisynthetic diet (Complan) lost less weight post-operatively than those maintained on the pelleted diet (Fig. 1), and showed a more rapid recovery of food intake. No significant distension of the abdomen was seen in vagotomized rats fed the semisynthetic diet (stomach girth: control 48±4 mm, vagotomized 56±4 mm, n.s.), nor was there any significant difference in the weight of gastric contents between the control (1·99±0·32 g) and vagotomized groups (2·13±0·72 g, n.s.) when measured at the end of the experiment. Antral weights were similar for control (0·91±0·03 g) and vagotomized rats (1·07±0·10 g, n.s.) but the mass of the forestomach was slightly increased by vagotomy (control 0·40±0·02 g, vagotomized 0·49±0·02 g, $P < 0·05$). Metabolizable energy intake was very similar for control and vagotomized rats (Table 3), because of pair-feeding, but once again the pattern of intake was noticed to be slightly altered, with controls consuming a greater proportion of their food during the light phase (08.00–20.00 h). Final body weight, weight gain, and carcass energy retention were all significantly elevated in vagotomized compared to control rats (Table 3 and Fig. 1) but energy expenditure did not differ between the two groups. Gross energetic efficiency was slightly decreased and net efficiency significantly reduced in vagotomized animals.

Resting $\dot{V}_{O_2}$ before gastric feeding (Table 4) was not affected by vagotomy or insulin injections. Feeding a single meal of the semisynthetic diet stimulated $\dot{V}_{O_2}$ by 17% in control rats, but by only 9·5% in vagotomized animals. Insulin injection enhanced

the thermic effect of food only in vagotomized animals, such that this no longer differed from the response seen in controls. Interscapular b.a.t. mass, protein content and mitochondrial protein were unaffected by vagotomy (Table 4), but specific GDP binding was decreased by 35%.

TABLE 3. Energy balance of control and vagotomized rats fed the semisynthetic diet (Expt. 2)

	Control	Vagotomized
Final body wt. (g)	257±6	283±8*
Metabolizable energy intake (kJ)	3980±80	4270±95 N.s.
(kJ/kg$^{0.75}$.day)	595±10	615±5 N.s.
Body energy gain (kJ)	380±45	570±45*
Energy expenditure (kJ)	3600±45	3700±60 N.s.
(kJ/kg$^{0.75}$.day)	540±10	530±5 N.s.
Gross energetic efficiency (%)	9·5±1·2	13·3±2·3 N.s.
Net efficiency (%)	32±6	42±3**

Mean values±S.E. of mean. *$P < 0.05$, **$P < 0.01$ compared to controls. N.s., not significantly different.

TABLE 4. Oxygen consumption and brown fat of control and vagotomized rats fed the powdered semisynthetic diet

	Control	Vagotomized
Resting $\dot{V}_{O_2}$ (ml/kg$^{0.75}$.min)		
No treatment		
Before meal	14·33±0·43	14·15±0·36 N.s.
After meal	16·76±0·65	15·49±0·33 N.s.
Increase (%)	16·7±2·1	9·5±1·1**
Insulin treatment		
Before meal	14·02±0·31	14·92±0·33 N.s.
After meal	16·09±0·34	17·04±0·44 N.s.
Increase (%)	14·8±1·1	14·2±1·0 N.s.
Brown adipose tissue		
Interscapular b.a.t. mass (mg)	226±14	204±17 N.s.
Protein content (mg)	13·5±0·8	11·8±1·3 N.s.
(%)	6·1±0·5	5·6±0·5 N.s.
Mitochondrial protein (mg)	1·58±0·07	1·35±0·12 N.s.
Specific GDP binding (pmol/mg protein)	34±3	22±2**

Data from Expt. 2. Effect of a single meal on oxygen consumption of control and vagotomized rats with or without insulin treatment, and brown adipose tissue mass and activity at the end of the experiment. All animals were fed a powdered diet (Complan).

**$P < 0.01$ compared to controls. N.s., not significantly different.

(d) The effects of chronic insulin treatment on vagotomized rats (Expt. 3)

In this experiment, body weight gain was slightly increased in vagotomized rats fed the semisynthetic diet (107±4 g) compared to the sham-operated controls (94±3 g, $P < 0.05$) in spite of almost identical levels of energy intake (control 4670±55 kJ, vagotomized 4565±85 kJ). Chronic insulin injections did not significantly affect energy intake (sham 4675±150 kJ, vagotomized 4685±180 kJ), and weight gain was similar for sham (84±7 g) and vagotomized animals (92±5 g, n.s.).

Specific GDP binding to isolated brown fat mitochondria was reduced in vagotomized rats (45 ± 4 pmol/mg protein) compared to sham operated (62 ± 4 pmol/mg protein, $P < 0.01$). Chronic insulin treatment stimulated GDP binding in both groups, but abolished the difference between sham-operated and vagotomized rats (sham plus insulin 79 ± 5 pmol/mg protein, vagotomized plus insulin 82 ± 8 pmol/mg protein, n.s.).

DISCUSSION

Varying criteria have been used to assess the success of subdiaphragmatic vagotomy, such as decreased gastric acid secretion, microscopic examination of the gastro-oesophageal region and gastric distension (e.g. Sawchenko, Gold & Ferrazano, 1977; Gold, Sawchenko, de Luca, Alexander & Eng, 1980; King *et al.* 1980). In the present study we used the absence of a change in intra-gastric pressure in response to cervical vagal stimulation as an indication that the subdiaphragmatic vagotomy was successful. Whilst this test demonstrates that the vagal efferent supply to the gastric muscle has been functionally destroyed, we do not have any monitor of the state of the abdominal vagal afferent innervation. However, as the abdominal vagal trunks contain both afferents and efferents (Gabella & Pease, 1973), it is likely that the surgery used in this study will have interrupted both types of fibre. It should be borne in mind that section of the abdominal vagal trunks will not only denervate the stomach but also the other abdominal organs supplied by these nerves, and hence contribute to the effects observed in this study. Insulin levels were not measured in these experiments, but blood glucose levels have been monitored in a few animals (N. J. Rothwell & M. J. Stock, unpublished observations) and were elevated in the vagotomized animals.

Acute gastric distension following section of the subdiaphragmatic vagi has often been used as the sole criterion for the completeness of the lesion (e.g. Ojeda, White, Aguado, Advis & Andersen, 1983). However, the results of this study show that the gastric distension is largely dependent on the type of diet used. The pelleted rodent diets used in this (Expt. 1, Table 1) and earlier studies are very poorly digestible and appear to result in severe gastric distension. In fact, many workers have observed prolonged and dramatic weight losses and a high mortality following vagotomy (e.g. Gold *et al.* 1980; King *et al.* 1980). The semisynthetic, powdered diet used in Expts. 2 and 3 was of much higher metabolizable energy density so that normal energy intakes could be achieved with a lower mass of food, and although food intake was lower than that normally seen in free-feeding intact animals, post-operative weight losses were small.

These results suggest that gastric distension (and hypertrophy) may be a poor index of the success of gastric vagotomy in animals maintained on high-quality diets. In addition, they reiterate and emphasize the fact that reductions in food intake (and hence body weight) following vagotomy may result simply from severe stomach distension, particularly with poorly digestible diets of low metabolizable energy density. This may provide part of the explanation for the differential effects of vagotomy on hypothalamic obesity or dietary obesity (e.g. Powley & Opsahl, 1974; Inoue & Bray, 1977; King *et al.* 1978; Wampler & Snowdon, 1979; Gold *et al.* 1980),

and questions the validity of experiments in which neither food intake nor gastric distension were measured, or the type of diet reported (e.g. King *et al.* 1980; Gold *et al.* 1980).

Vagotomized rats fed either pelleted or powdered diets had a lower energy expenditure and/or greater efficiency of energy utilization than pair-fed controls. These differences in energy balance were probably attenuated by the altered meal pattern of control animals, since this diurnal 'meal-feeding' pattern of intake increases fat deposition and energetic efficiency (Fabry, 1969), particularly in nocturnal 'nibbling' animals, such as the rat. Furthermore, because of this pattern of food consumption, control rats had consumed no solid food for almost 24 h before the measurements of $\dot{V}_{O_2}$, and this would in itself tend to depress metabolic rate, whereas the vagotomized animals were eating up to 14 h before (the time when solid food was removed), and probably had some food remaining in their stomachs at the time of the measurements. Digestibility was not measured in the first experiment, but in the second experiment (semisynthetic), all faeces and urine were collected and analysed. Thus, differential energy losses in waste products could not have accounted for the enhanced energetic efficiency of vagotomized rats.

Vagotomized rats showed normal thermogenic responses to noradrenaline (Table 2) indicating that peripheral thermogenic mechanisms (probably involving b.a.t.) remain intact. The rise in metabolic rate after intubation with fat was also similar for control and vagotomized rats, but thermic responses to carbohydrate and the mixed nutrient semisynthetic meal were both depressed by vagotomy, and normalized by acute insulin treatment. Interestingly, thermic responses to carbohydrate were depressed by 60 %, in vagotomized rats but the response to the mixed-nutrient meal by only 45 %. This could be due to the fact that the latter contains 32 % lipid and this, together with the normal responses seen after ingestion of fat, suggests that feeding vagotomized rats a very high fat diet might normalize their energetic efficiency. These differential effects of fat and carbohydrate on $\dot{V}_{O_2}$ probably reflect the involvement of insulin, which is essential for the thermic response to carbohydrate but apparently not required for the effects of fat.

The activity of the proton conductance pathway in b.a.t. mitochondria, assessed from GDP binding, was significantly reduced in vagotomized rats consuming pelleted or powdered diets. This was in spite of the restricted food intake and 'meal-feeding' pattern of intake in control animals, both of which tend to depress b.a.t. activity (Rothwell & Stock, 1982*a*). The reduced energy expenditure and thermic responses, and enhanced energetic efficiency of vagotomized animals may therefore be due partly to lower thermogenesis in b.a.t. Chronic treatment of rats with insulin (Expt. 3) prevented the reduction in brown fat activity (i.e. mitochondrial GDP binding) associated with vagotomy, and in intact animals, insulin was found to stimulate GDP binding, a finding which agrees with previous work (Seydoux, Trimble, Bouillaud, Assimacopoulos-Jeannet, Bas, Riquier, Giacobino & Girardier, 1984). These results indicate that impairments in thermogenesis and b.a.t. activity following vagotomy are likely to be due to the reduction in insulin levels, and that increased levels of the hormone can stimulate brown fat thermogenesis.

The results of this study demonstrate the importance of diet composition and digestibility on the responses to vagotomy. They also represent the first attempts to

measure energy balance and thermogenesis in vagotomized animals. The data show that vagotomy depresses thermogenic responses to carbohydrate, lowers b.a.t. activity and enhances energetic efficiency. This results in greater rates of body gain than in pair-fed controls consuming isoenergetic amounts of food. These effects appear to be due largely to impaired insulin release, since they can be reversed by acute or chronic treatment of vagotomized rats with insulin.

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REFERENCES

ANDREWS, P. L. R. & SCRATCHERD, T. (1980). The gastric motility patterns induced by direct and reflex excitation of the vagus nerves in the ferret. *Journal of Physiology* **302**, 363–378.

BRAY, G. A. & YORK, D. A. (1979). Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiological Reviews* **59**, 719–809.

BROOKS, S. L., ROTHWELL, N. J. & STOCK, M. J. (1982). Effects of diet and acute noradrenaline treatment on brown adipose tissue development and mitochondrial purine nucleotide binding. *Quarterly Journal of Experimental Physiology* **67**, 259–268.

COX, J. E. & POWLEY, T. L. (1981). Prior vagotomy blocks VMH obesity in pair-fed rats. *American Journal of Physiology* **240**, E573–583.

FABRY, P. (1969). *Feeding Patterns and Nutritional Adaptations*. London: Butterworths.

GABELLA, G. & PEASE, H. L. (1973). Number of axons in the abdominal vagus of the rat. *Brain Research* **58**, 465–469.

GOLD, R. M., SAWCHENKO, P. E., DE LUCA, C., ALEXANDER, J. & ENG, R. (1980). Vagal mediation of hypothalamic but not of supermarket dietary obesity. *American Journal of Physiology* **238**, R447–453.

INOUE, S. & BRAY, G. A. (1977). The effects of subdiaphragmatic vagotomy in rats with ventromedial hypothalamic obesity. *Endocrinology* **100**, 108–114.

INOUE, S., BRAY, G. A. & MULLEN, Y. S. (1978). Transplantation of pancreatic β-cells prevents development of hypothalamic obesity in rats. *American Journal of Physiology* **238**, E266–271.

JEANRENAUD, B. (1978). An overview of experimental models of obesity. In *Recent Advances in Obesity Research – II*, ed. BRAY, G. A., pp. 111–122. London: Newman.

KING, B. M., CARPENTER, R. G., STAMOUTSOS, B. A., FROHMAN, L. A. & GROSSMAN, S. P. (1978). Hyperphagia and obesity following ventromedial hypothalamic lesions in rats with subdiaphragmatic vagotomy. *Physiology & Behaviour* **20**, 643–651.

KING, B. M., PHELPS, G. R. & FROHMAN, L. A. (1980). Hypothalamic obesity in female rats in absence of vagally mediated hyperinsulinaemia. *American Journal of Physiology* **239**, E437–441.

LANDSBERG, L. & YOUNG, J. B. (1983). Autonomic regulation of thermogenesis. In *Mammalian Thermogenesis*, ed. GIRARDIER, L. & STOCK, M. J., pp. 99–140. London: Chapman & Hall.

NICHOLLS, D. G. & LOCKE, R. (1984). Thermogenic mechanisms in brown fat. *Physiological Reviews* **64**, 1–64.

OJEDA, S. R., WHITE, S., AGUADO, L. I., ADVIS, J. P. & ANDERSEN, J. M. (1983). Abdominal vagotomy delays the onset of puberty and inhibits ovarian function in the female rat. *Neuroendocrinology* **36**, 261–267.

POWLEY, T. L. (1977). The ventromedial hypothalamic syndrome satiety and a cephalic phase hypothesis. *Physiological Reviews* **84**, 89–126.

POWLEY, T. L. & OPSAHL, C. A. (1974). Ventromedial hypothalamic obesity abolished by subdiaphragmatic vagotomy. *American Journal of Physiology* **226**, 25–33.

ROTHWELL, N. J., SAVILLE, M. E. & STOCK, M. J. (1981). Acute effects of food, 2-deoxy-D-glucose and noradrenaline on metabolic rate and brown adipose tissue in normal and atropinised lean and obese (fa/fa) Zucker rats. *Pflügers Archiv* **392**, 172–177.

ROTHWELL, N. J. & STOCK, M. J. (1981). Regulation of energy balance. *Annual Review of Nutrition* **1**, 235–256.

ROTHWELL, N. J. & STOCK, M. J. (1982*a*). Effect of chronic food restriction on energy balance, thermogenic capacity, and brown adipose tissue activity in the rat. *Bioscience Reports* **2**, 543–549.

ROTHWELL, N. J. & STOCK, M. J. (1982*b*). Effects of feeding a palatable 'cafeteria' diet on energy balance in young and adult lean (+/?) Zucker rats. *British Journal of Nutrition* **47**, 461–471.

ROTHWELL, N. J. & STOCK, M. J. (1982*c*). Energy expenditure of 'cafeteria-fed' rats determined from measurements of energy balance and direct calorimetry. *Journal of Physiology* **328**, 371–377.

ROTHWELL, N. J. & STOCK, M. J. (1983). Diet-induced thermogenesis. In *Mammalian Thermogenesis*, ed. GIRARDIER, L. & STOCK, M. J., pp. 208–233. London: Chapman & Hall.

ROTHWELL, N. J. & STOCK, M. J. (1984). Brown adipose tissue. In *Recent Advances in Physiology* – 10, ed. BAKER, P. F., pp. 208–233. Edinburgh: Churchill Livingstone.

ROTHWELL, N. J., STOCK, M. J. & WARWICK, B. P. (1984). Involvement of insulin in the acute thermogenic responses to food and non-metabolisable substances. *Metabolism* (in the Press).

ROWLAND, N. & ENGLE, D. J. (1978). Hypothalamic hyperphagia prevented by prior subdiaphragmatic vagotomy. Insulin hyperphagia is unaffected. *Physiology & Behaviour* **21**, 685–689.

SAWCHENKO, P. E., GOLD, R. M. & FERRAZANO, P. A. (1977). Abolition by selective vagotomy of the influence of water temperature on intake: Mediation via enhanced gastric clearance. *Physiology & Behaviour* **17**, 461–471.

SEYDOUX, J., ROHNER-JEANRENAUD, F., ASSIMACOPOULOS-JEANNET, F., JEANRENAUD, B. & GIRARDIER, L. (1981). Functional disconnection of brown adipose tissue in hypothalamic obesity in rats. *Pflügers Archiv* **390**, 1–4.

SEYDOUX, J., TRIMBLE, E. R., BOUILLAUD, F., ASSIMACOPOULOS-JEANNET, F., BAS, S., RIQUIER, D., GIACOBINO, J. P. & GIRARDIER, L. (1984). Modulation of β-oxidation and proton conductance pathway of brown adipose tissue in hypo and hyperinsulinaemic states. *FEBS Letters* **166**, 141–145.

SLINDE, E., PEDERSON, J. J. & FLATMARK, T. (1975). Sedimentation coefficient and buoyant density of brown adipose tissue mitochondria from guinea pigs. *Analytical Biochemistry* **65**, 581–585.

STOCK, M. J. (1975). An automatic, closed-circuit oxygen consumption apparatus for small animals. *Journal of Applied Physiology* **39**, 849–850.

TRAYHURN, P. & JAMES, W. P. T. (1983). Thermogenesis and obesity. In *Mammalian Thermogenesis*, ed. GIRARDIER, L. & STOCK, M. J., pp. 234–258. London: Chapman & Hall.

WAMPLER, R. S. & SNOWDON, C. T. (1979). Development of VMH obesity in vagotomized rats. *Physiology & Behaviour* **22**, 85–93.

YORK, D. A. & BRAY, G. A. (1972). Dependence of hypothalamic obesity on insulin, the pituitary and the adrenal gland. *Endocrinology* **90**, 885–894.