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THE RESPONSE OF THE PANCREAS OF THE ANAESTHETIZED CAT TO SECRETIN BEFORE, DURING AND AFTER REVERSIBLE VAGAL BLOCKADE

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SUMMARY

1. Cooling the cervical vagi of the anaesthetized splanchnectomized cat to 2 °C caused a 54·4 ± 8·8 % inhibition of pancreatic electrolyte secretion stimulated submaximally with pure secretin.

2. On rewarming the vagi there was a prolonged increase in secretion rate over and above the control rate which existed before cooling. The increase lasted about 90 min.

3. There were no changes in acid/base status due to interference of the lung inflation reflex which could account for the inhibition of secretion and the subsequent rebound.

4. Cold block of the cervical vagi increased the transpancreatic electrical conductance, indicating that vasodilation had occurred and therefore eliminated a vasomotor cause for the inhibition.

5. Electrolyte secretion was also inhibited by bilateral vagal section.

6. Atropine only partially prevented the inhibitory response to vagal cooling. A cholinergic mechanism, therefore, accounted for some but not all of the response to vagal cooling.

7. It is concluded that even in the fasted, anaesthetized animal vagal impulses facilitate the action of secretin on the pancreas. This facilitation is only partially cholinergic; the major part of the response is due to some non-cholinergic transmitter substance. Such a mechanism may be necessary to potentiate the action of the very small amounts of secretin which appear to be released during a meal.

INTRODUCTION

Electrical excitation of the abdominal vagus nerves of the anaesthetized cat stimulates mainly enzyme secretion, whereas the effect on fluid secretion is small (Korovitsky, 1923; Sergeyeva, 1938; Harper & Vass, 1941; Brown, Harper & Scratcherd, 1967). However, Lenninger & Ohlin (1971) were able to obtain a small secretion after perfusing the duct system with 0·9 % NaCl after a latency which varied from 1 to 3 min. Experiments of this nature have led to the view that secretion of fluid and bicarbonate is largely a function of hormones, particularly secretin (Wang & Grossman, 1951). With the establishment of sensitive, specific and reliable methods

of radioimmunoassay of secretin (Shaffalitzky de Muckadell & Fahrenkrug, 1977, 1978) the dominance of secretin as the major stimulant of water and electrolyte secretion appeared less secure. The concentration of secretin in the peripheral blood increased to about 6 p-mole/l. and this only in bursts, and it is questionable that these levels could account for the volume response to a meal. Indeed when infused continuously in man to achieve steady concentrations of this magnitude the output from the pancreas was less than 5% of the total secretory capacity of the gland (Hacki, Greenberg & Bloom, 1978). It is therefore likely that considerable potentiation of the action of secretin occurs due to the simultaneous action of pancreozymin and vagal activity. This paper deals with the interaction between the vagus and secretin and examines the possible mechanisms which may or may not operate.

METHODS

Experiments were performed on seventeen cats of either sex fasted for 12–18 hr but allowed access to water. Anaesthesia was induced with ether and maintained with intravenous chloralose (37·5 mg/kg) and urethane (450 mg/kg). The trachea was intubated through the mouth and, from this point onwards in most experiments, bicarbonate was infused continuously to replace that lost in the pancreatic juice and thereby prevent acidaemia from developing. The splanchnic nerves were sectioned extraperitoneally after which the pylorus was occluded by ligature. The pancreatic duct was cannulated at the point it pierced the duodenal wall and held in place by a ligature which also included the bile duct. In some experiments plane-parallel platinum electrodes on a Perspex frame were placed across the tail of the pancreas and connected to a Wayne Kerr Bridge B221 (Clarke, Greenwell, Harper, Sankey & Scratcherd, 1967) to measure the electrical impedance at 1592 Hz. The vago-sympathetic trunks were dissected free from the carotid arteries over a distance of 1·5 cm and placed on thermodes made from a 'Frigistor' cooling module (Mectron; De La Rue Ltd. Canal Estate, Langley, Bucks.) as described by Linden, Mary & Weatherill (1981). Foam rubber, 3 mm thick, was then placed between the thermode and the underlying tissue to insulate against heat loss. The temperature of the vagi were monitored with thermocouples. The femoral artery was cannulated and both arterial blood samples and blood pressure were taken from this vessel. Pancreatic secretion was stimulated by continuous intravenous infusion of either pure porcine secretin (Karolinska Institute) or synthetic secretin at rates between 0·34 and 0·84 c.u./kg per hour to give secretory rates of approximately one-third to one-half maximum. Pancreatic juice was collected in small tared vessels and the output expressed as g/10 min. Sodium and potassium concentrations were determined on a Corning Eel flame photometer, chloride on a Buchler Chloridometer and protein by a microbiuret method. Bicarbonate secretion was calculated from the relationship $[Na]+[K]-[Cl]=[HCO_3]$. In twenty-six samples of pancreatic juice Na, K, Cl and HCO_3 were directly estimated (HCO_3 by Natelson microgasometer) and the above relationship was shown to be a reasonable approximation, there being a deficit of cation of $8·3 \pm 0·2$ m-mole/l., which could be partially accounted for by the Ca^{2+} and Mg^{2+} content of the juice. The error in estimating bicarbonate by this method varied from 5 to 7%. All results are expressed as the mean (±S.E. of mean), with the individual determinations in parentheses. Paired sample t tests were used to assess significance.

RESULTS

The effect of vagal block on volume of water and electrolyte secretion

Pancreatic electrolyte secretion was stimulated in nine animals by intravenous infusion of pure porcine or synthetic secretin. When submaximal steady rates were established, the vagus nerves in the neck were cooled down to 2 °C. This procedure resulted in the inhibition of electrolyte secretion from a mean of $0·88 \pm 0·08$ g/10 min to $0·41 \pm 0·08$ g/10 min ($P < 0·001$) which was the mean of the lowest secretion

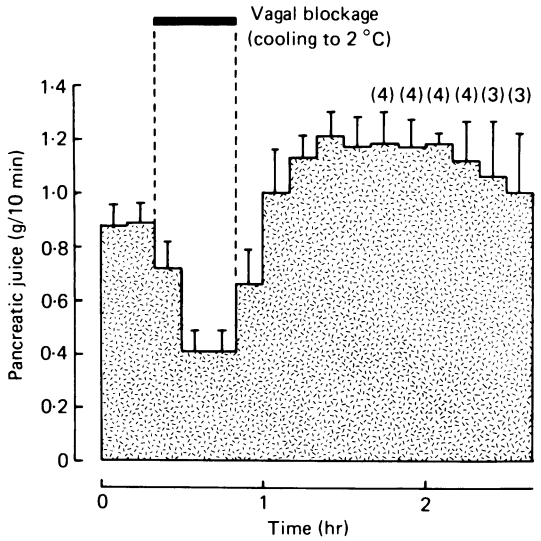

Fig. 1. The inhibition of pancreatic secretion caused by cooling the cervical vagi to 2 °C. The duration of cooling is indicated by the filled bar. Secretion was stimulated submaximally by the continuous infusion of pure porcine secretin. Bars represent ±S.E. of the mean, for nine experiments except where indicated in parentheses.

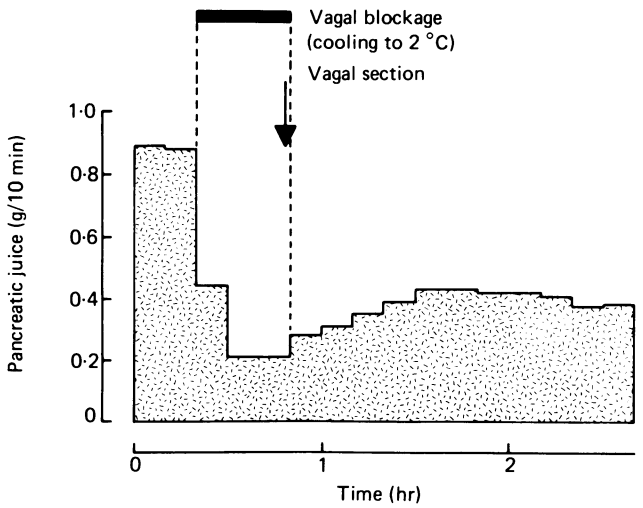

Fig. 2. The effect of bilateral vagal section on the response of the pancreas to secretin. The cervical vagi were cooled to 2 °C over the period indicated by the filled bar. One minute before rewarming both vagi were sectioned central to the block. Note some partial recovery.

occurring in the second or third 10 min period of cooling (Fig. 1). On rewarming the nerves, not only did full recovery of the secretory response occur, but there was an increase over the control rate of secretion which reached a maximum of $1{\cdot}17 \pm 0{\cdot}12$ g/10 min. ($P < 0{\cdot}001$) 30–40 min after rewarming commenced. This increase in secretion rate was prolonged, usually lasting longer than an hour after

re-establishment of vagal conduction and in one case the secretion rate did not return to control values for over 2 hr. The inhibition remained if the vagus nerves were sectioned central to the thermodes just before the end of the cooling period (Figs. 2 and 6).

During the cooling period there was no significant change in either the sodium or potassium concentration in the pancreatic juice, although the concentration of chloride increased and that of bicarbonate fell (Fig. 3). This increase could be accounted for by the fall in secretion rate during the inhibition.

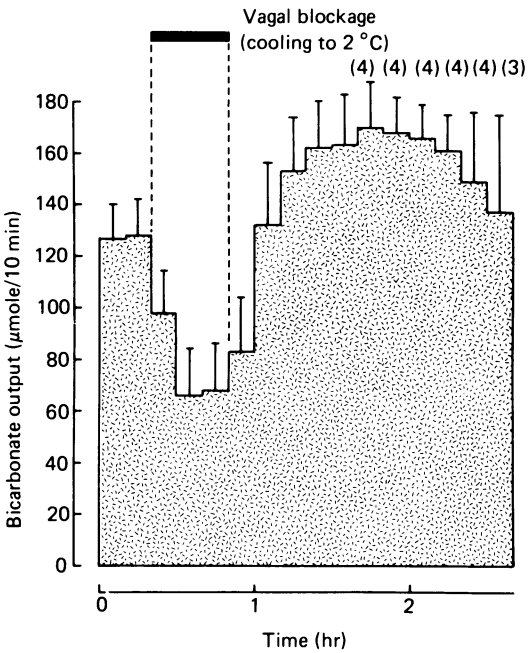

Fig. 3. The inhibition of bicarbonate output caused by cooling the cervical vagi for the experiments illustrated in Fig. 1.

The inhibition of secretion described above could be due to a number of factors, each of which was investigated separately. Lowering of body temperature is known to reduce pancreatic secretion rate (Osborne & Greengard, 1941) but no changes in temperature were recorded from a thermistor placed in direct contact with the pancreas. There was, however, a fall in temperature of $0{\cdot}42 \pm 0{\cdot}1$ °C ($n = 10$) recorded, after 30 min cooling, by a thermistor situated in the oesophagus immediately below the cooling thermodes. However, the inhibition was established within 10 min before any temperature change was recorded and persisted after vagal section even though the temperature had returned to normal (Fig. 2).

Changes in acid/base status

Pancreatic secretion is sensitive to both the pH and bicarbonate concentration of the plasma, so that by lowering either or both parameters pancreatic secretion is inhibited (Rawls, Wistrand & Maren, 1963; Pak, Hong, Pak & Hong, 1966; Case, Scratcherd & Wynne, 1970; Case, Hotz, Hutson, Wynne & Scratcherd, 1979).

Blockade of the cervical vagus produces respiratory changes by interruption of the lung inflation reflex and thereby could produce a disturbance of acid/base status. In twelve experiments the pH and bicarbonate concentration of arterial blood were measured before and during vagal blockade by cooling the cervical vagus. The acid/base status in three of the animals was uncontrolled, so that they became acidotic, with an arterial pH before block of $7{\cdot}256 \pm 0{\cdot}009$ and bicarbonate concentration of $13{\cdot}5 \pm 2{\cdot}4$ mM, and during blockage a pH of $7{\cdot}259 \pm 0{\cdot}02$ and bicarbonate concentration of $13{\cdot}2 \pm 3{\cdot}7$ mM. There was thus very little change, whereas the secretion rate fell from a mean of $0{\cdot}995 \pm 0{\cdot}13$ g/10 min to $0{\cdot}40 \pm 0{\cdot}17$ g/10 min during the second 10 min period of vagal blockade.

In nine other animals the acidotic state was prevented by intravenous infusions of bicarbonate. In these animals the arterial pH and bicarbonate concentrations before blockade were $7{\cdot}387 \pm 0{\cdot}03$ and $22{\cdot}9 \pm 0{\cdot}79$ mM respectively, whereas during blockade the corresponding figures were $7{\cdot}414 \pm 0{\cdot}02$ for pH and $23{\cdot}5 \pm 1{\cdot}12$ mM for bicarbonate. The secretion rate during the latter experiments fell from $0{\cdot}984 \pm 0{\cdot}20$ g/10 min before vagal block to $0{\cdot}447 \pm 0{\cdot}18$ g/10 min during blockade.

Vasomotor effects on the pancreas during vagal blockade

Pancreatic secretion can be profoundly affected by vasomotor reactions, inhibition occurring with vasoconstriction (Barlow, Greenwell, Harper & Scratcherd, 1974) and therefore it is important to eliminate vasomotor effects as a cause of inhibition of pancreatic secretion.

Direct measurement of pancreatic blood flow is not possible without considerable surgery and therefore damage to the vagal nerve supply of the gland. To circumvent this difficulty and to cause as little disturbance to the gland as possible the transverse electrical conductance across the tail of the pancreas was measured at a frequency of 1592 Hz, as an index of vasomotor changes. Barlow *et al.* (1974) had noted a striking parallelism between the time course of the biphasic changes in pancreatic blood flow and transverse electrical conductance across the tail of the pancreas in response to the intravenous injection of catecholamines and concluded that the method was a sensitive detector of blood flow and blood flow changes. The rationale for using this technique was that the pancreas can be regarded as a suspension of non-conducting cells in an electrolyte solution of low resistivity (Clark *et al.* 1967). As vasomotor changes alter the ratio of e.c.f. to cells in the pancreatic tissue, this would lead to an increase in conductance with vasodilation and a decrease with vasoconstriction, as the specific resistance of cat whole blood of 136·9 Ω cm at 1592 Hz and 38·5 °C is considerably less than that of pancreatic tissue which is 820 Ω cm (Clark *et al.* 1967).

As this method is difficult to interpret when secretion rate is changing, the electrical conductance was measured before, during and after vagal blockade in the absence of secretin stimulation. In six experiments on three animals vagal cooling was always associated with an increase in conductance and hence an increase in blood flow and/or blood content (Fig. 4). As the blood pressure increased simultaneously, both before and after atropine (Fig. 5), the effect could be passive as a consequence of increased perfusion pressure. Therefore the vasomotor changes could not account for the observed inhibition of pancreatic secretion.

The action of atropine

The effect of atropine, at doses of 0·1 and 1·0 mg/kg body weight, was investigated under two experimental conditions. When vagal block was established atropine was slowly injected intravenously 1 min before the end of the cooling period, when pancreatic secretory inhibition was at or near maximum. The secretion rate slowly

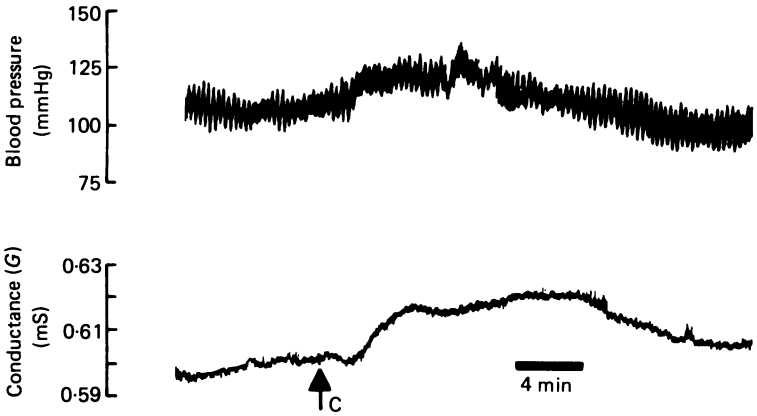

Fig. 4. The effect of bilateral cervical vagal blockade on the blood pressure (above) and trans-pancreatic electrical conductance (below). At C the vagi were cooled to 2 °C for 15 min.

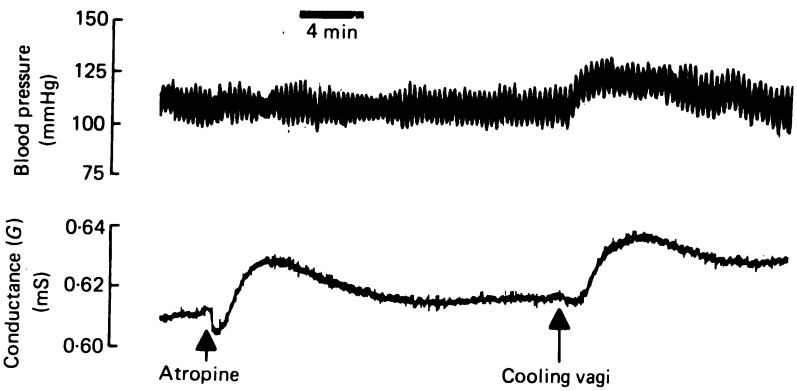

Fig. 5. The effects on the blood pressure (above) and trans-pancreatic electrical conductance (below) of atropine (on the left) and cooling both cervical vagi (on the right). The duration of cooling period was 10 min from the respective arrows.

increased when the vagal block was removed, but never returned to control levels. When atropine was injected before vagal block was established, inhibition of secretion still occurred when the vagi were cooled, but the response was not of the same magnitude as that which occurred in the absence of atropine (Fig. 6). Whereas pancreatic secretion was inhibited by $53{\cdot}4 \pm 8{\cdot}8\,\%$ by vagal block in the absence of atropine, in its presence the inhibition was $38{\cdot}3 \pm 5{\cdot}9\,\%$ ($n = 6$). Thus a cholinergic mechanism accounts for some but not all of the response. This suggests that the response is due to interruption of conduction in a non-cholinergic pathway.

The effect of vagal blockade on spontaneous protein secretion

The pancreatic juice stimulated by pure secretin always contains some protein though the amounts are small. In thirteen experiments vagal blockade by cooling caused a reduction in protein output from $0{\cdot}7 \pm 0{\cdot}15$ to $0{\cdot}53 \pm 0{\cdot}13$ mg/10 min, a difference which did not reach statistical significance. In five of these experiments there was a marked reduction in output on cooling the vagi, but in the remainder no change was observed.

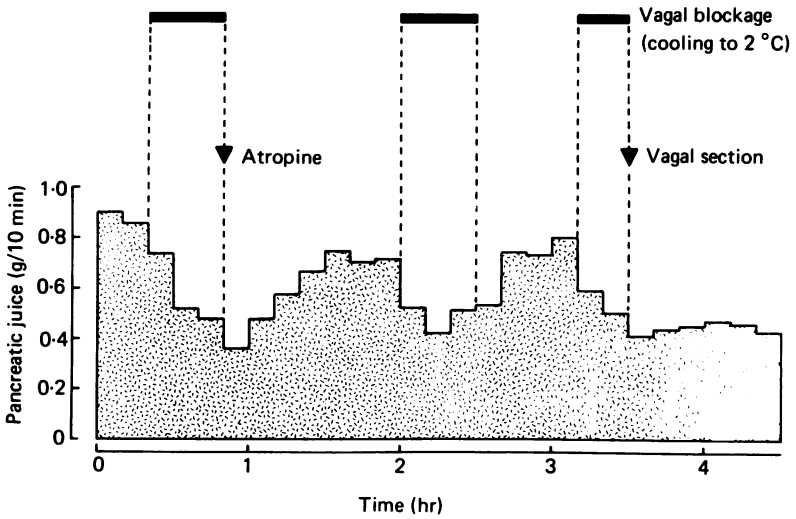

Fig. 6. The effect of cooling the cervical vagi to 2 °C for the duration indicated by the filled bars on pancreatic secretion stimulated sub-maximally with pure secretin, before and after the intravenous injection of atropine. Atropine was administered 1 min before the cooling period was terminated (first cooling period). Note that recovery after atropine was not complete, but cold block of the vagi was still effective (second cooling period). One minute before the end of the third cooling period, both vagi were sectioned central to the thermodes. Note there was no immediate recovery.

DISCUSSION

In this paper vagal block was produced by cooling the cervical vagi, a procedure which has the advantage of being repeatable many times in the same animal whereas vagotomy can be carried out once only. Reversible block allows observations to be made not only on the consequences of the block, but also on the effects which occur on restoration of nervous conduction. It also allows, within one experiment, the effects of other agents to be tested in the presence and absence of a functional vagal innervation.

The contribution of the vagus nerve to the response of the pancreas to secretin has been assessed by removing the vagal influence. The effect of truncal vagotomy on pancreatic secretion stimulated by exogenous secretin has produced conflicting results. After vagotomy the response to secretin in dogs was unchanged (Henriksen, 1969; Konturek, Becker & Thompson, 1974; Debas, Konturek & Grossman, 1975),

decreased (Magee, Fragola & White, 1965; Lenninger, Magee & White, 1965), or increased (Tankel & Hollander, 1958; Moreland & Johnson, 1971). In man a significant decrease in the response to secretin has been reported (Wormsley, 1972) whilst in anaesthetized cats the response was either decreased or unchanged (Harper & Vass, 1941). The reasons for these differences are not clear, but may be related to the experimental techniques, the type of fistula used and the method of collecting pancreatic juice, the purity of the secretin used and the method of its administration (by bolus or continuous infusion (at maximal or submaximal rates)) and the length of time which had elapsed since the vagotomy was performed.

In the experiments reported in this paper temporary blockade of vagal conduction inhibited pancreatic secretion, an effect which was followed by a prolonged increase in the sensitivity of the gland to secretin on restoration of conduction. After bilateral vagotomy performed towards the end of the cooling period a small recovery was noted, whereas after the same procedure in the presence of atropine no recovery was observed. This might suggest that acetylcholine is in some way involved in the regulation of the sensitivity of the gland. There are several possible causes which could account for the inhibitory effect of vagal blockade, some acting directly on the secretory cell, others acting indirectly. Possible indirect effects by interference with acid/base balance consequent upon respiratory changes have been referred to in the text, and it has been concluded that they could not account for either the inhibition or the rebound of secretion. Cold block could imitate an 'unloading' or aortic baro- and chemoreceptors with a resultant activation of abdominal sympathetic discharge in the splanchnic nerves, consequently inhibiting secretion by a direct action on the secretory cell or indirectly by vasoconstriction (Barlow *et al.* 1974). However, inhibition from such a cause would be unlikely as it occurred after splanchnectomy. Evidence that the splanchnectomy was effective is given by the increase in trans-pancreatic electrical conductance which occurred on cooling the vagi, indicating that vasodilation had occurred and therefore a vasomotor cause for the inhibition was unlikely.

The most plausible explanation would be that vagal block prevented the potentiation of secretin action by vagal transmitter substances. There may be at least three candidates, acetylcholine and possibly vasoactive intestinal polypeptide and substance P. There is good evidence for the presence of a post-ganglionic peptidergic innervation both for VIP and substance P (Sundler, Alumets, Hakenson, Fahrenkrug & Schaffalitzky de Muckadell, 1978); Hokfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygards & Pernow, 1977). The VIP neurones are related to the pancreatic duct, a major source of electrolyte (Schulz, Yamagata & Weske, 1969) and both VIP and substance P are effective in stimulating electrolyte secretion (Said & Mutt, 1972; Case, Smith & Scratcherd, 1976; Konturek, Radechi & Pucher, 1976; Thulin & Holm, 1977). Also Brown, Harper & Scratcherd (1963, 1967) have shown that the action of secretin could be potentiated by the vagus and this was resistant to atropine. Clearly both acetylcholine and non-cholinergic transmitters are involved, as atropine reduced but did not prevent the effects of vagal blockade. Such an explanation would imply that, in the basal state, efferent vagal activity was of such a magnitude as to be able to potentiate the action of secretin. In the anaesthetized rat, dog and ferret (and presumably cat) a proportion of efferent vagal fibres to the abdomen are active

(Davison & Grundy, 1978; Andrews, Fussey & Scratcherd, 1980; Grundy, Salih & Scratcherd, 1981) and might be sufficient to potentiate the action of secretin under the conditions of the experiments reported here. In the course of a meal, however, this activity would be considerably enhanced. Such a hypothesis is attractive, for the release of secretin is quite small and, although capable of stimulating a small flow of pancreatic juice (Schaffalitzky de Muckadell, Fahrenkrug, Watt-Boolson & Worning, 1978), may not be adequate for the needs of digestion. However, much larger amounts of juice would be secreted with the increase in vagal activity and pancreozymin release during the course of a meal, potentiating the action of secretin. There is a possible further alternative and this may be that as digestion comes to an end, signalled by a reduction in efferent discharge, then some humoral inhibitory agent could be liberated, which is, normally held in restraint by the vagus.

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