FEEDING AND CALCITONIN SECRETION IN SHEEP

M. PHILLIPPO, C. B. LAWRENCE, J. B. BRUCE AND D. R. DONALDSON
Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB

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SUMMARY
Calcitonin (CT) secretion was measured before, during and after feeding in three sheep with an exteriorized lobe of the thyroid gland. A significant ($P < 0.05$) increase in the rate of CT secretion occurred during the 15-min period immediately after the presentation of the food: this increase could be blocked by the local infusion of $35 \text{nmol} \cdot (\pm)\text{-propranolol}$. During the subsequent 165 min, the secretion of CT was not significantly altered from that before feeding. These results suggest that in the sheep there is an initial increase in CT secretion mediated via a $\beta$-adrenergic system and it is possibly associated with the excitement of receiving the food. In the remainder of the first 180 min after presentation of a feed, the rate of release was unaffected.

INTRODUCTION
Sheep show a remarkable series of physiological changes in response to the ingestion of food. Among these are the increased rates of excretion of calcium and of magnesium in urine (Stacy, 1969) which are associated with increased plasma calcium and magnesium levels, a lowered blood pH and a lowered plasma bicarbonate concentration. Since calcitonin (CT) is stimulated by an increased plasma calcium concentration, it seemed logical to study the release of this hormone during feeding and to elucidate its role, if any, in the process of calcium homeostasis after feeding.

MATERIALS AND METHODS
Three sheep, in which one lobe of the thyroid gland had been isolated together with its blood supply in a skin loop (Falconer, 1963) 18 months earlier, were used. The vascular arrangement of the isolated gland in the skin loop was examined radiographically before these experiments were started. Food was withheld from the animals during the 24 h preceding the actual experiment but water was given ad libitum. The animals were moved into metabolism cages on the morning of the experiment and catheters inserted into both jugular veins and into the carotid artery within the loop. Each animal was allowed to recover from any effects of these procedures before the experiment was started.

The cranial end of the loop was completely occluded at the start of the experiment and the blood flow through the thyroid gland was measured throughout the experiment.
by the intra-arterial infusion of tracer amounts of either unlabelled calcium chloride or of \(^{42}\)KCl in 0-9 % (w/v) sodium chloride solution. In six experiments no additional treatment was given; in a further three experiments, (±)-propranolol was added to the saline perfusate to give a concentration of 35 nmol/l in the blood perfusing the thyroid gland. Thyroid venous and peripheral blood samples were taken every 5 min for plasma calcium determinations; the remainder of each thyroid venous plasma sample was stored at -20 °C until assayed for calcitonin. After 30 min a feeding trough containing 350 g dried grass covered with 350 g of a barley diet was placed in front of the animal. Sampling was continued for a period of 60 min after the food was offered in most of the experiments but at intervals over a period of 180 min in two experiments. The results were analysed for successive 15-min periods, which in the ensuing discussion are referred to as A₁ and A₂ for those before feeding, and B₁, B₂, B₃ and B₄ for those after the start of feeding.

Calcitonin concentrations were assayed by the method of Leggate, Care & Frazer (1969), and plasma calcium concentrations were measured by the method of Gitelman (1967). Ultrafiltrable calcium concentrations in plasma were measured on samples taken anaerobically under mineral oil; the plasma was separated and centrifuged for 16 h at 8 °C through a Visking membrane in an atmosphere of 95 % CO₂ and 5 % O₂. Plasma pH was determined using a Radiometer E 5021 apparatus.

RESULTS

Eating pattern

In all the experiments the sheep ate the barley diet before starting to ingest the dried grass. The mean time before all the barley diet was eaten was 6·6 ± 0·3 min (n = 7; range 5–7 min). The mean length of the eating period was 42·6 ± 2·4 min (n = 7; range 35–59 min); the intensity of feeding was very rapid for the first 20–25 min, and then slowed during the last 15–20 min of feeding.

Hormonal secretion

In each of the four experiments without propranolol there was a significant increase in the CT secretion rate between periods A₂ and B₁ (see Table 1). This was due in the main to a very high release in the two samples taken directly after feeding. In only one of the experiments (71/31) did the secretion rate in periods subsequent to period B₁ remain significantly higher than periods A₁ and A₂ but in no experiment did it fall significantly below that before feeding. In two further experiments without propranolol the same initial changes were observed and the release of CT did not alter significantly during the 180 min following the start of feeding.

The addition of (±)-propranolol was effective in preventing the increased secretion rate seen in period B₁ in all three experiments (see Table 1). There was no significant alteration in the secretion rate of CT during any of the succeeding periods.

Plasma pH and ultrafiltrable calcium

The pH of the blood perfusing the thyroid gland in two experiments dropped from levels of 7·46 and 7·45 before feeding to 7·35 and 7·31 during feeding and remained at this decreased level during the whole of the experiment. In another experiment
Table 1. The effect of feeding sheep in the absence (control) or presence of 35 nM (±)-propranolol, on calcitonin secretion rate (X, μu./min) and plasma calcium concentration (Y, mequiv./l)

(A1, A2, etc. refer to periods before, during and after feeding. Means and standard errors are given for three values per period. Food was offered at the start of period B1.)

<table>
<thead>
<tr>
<th>Expt no.</th>
<th>Before feeding</th>
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<tr>
<td></td>
<td>A₁</td>
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<td>B₁</td>
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<tr>
<td>Control sheep 71/31</td>
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<td>104±2±11±9**</td>
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</table>

Significance of difference from the level present before feeding: † P < 0·1; * P < 0·05; ** P < 0·01; *** P < 0·001.
the percentage of ultrafiltrable calcium in the plasma rose from a level of 47.9 % before feeding to one of 58.1 % during eating; the total calcium concentrations were 5.80 and 5.85 mequiv. Ca/l; the CT release was 8.5 and 8.9 mu./min respectively.

-discussion-

The release of CT from the thyroid is dependent on the perfusing plasma calcium concentration which may operate by means of an adenyl cyclase system (Care, Bates & Gitelman, 1970). Drugs, such as glucagon and adrenaline, that activate the adenyl cyclase system are also effective in increasing the release of calcitonin (Care et al. 1970; Phillippo, Bruce & Lawrence, 1970; Avioli, Shieber & Kipnis, 1971). The effect of adrenaline can be blocked by the b-adrenergic blocking agent (±)-propranolol in the pig (Care et al. 1970), in the dog (Avioli et al. 1971) and in the conscious sheep (Phillippo et al. 1970).

Gray & Munson (1969) found that hypercalcaemia follows feeding in a fasted rat that has been thyroidectomized but that this effect is not seen in an intact rat, suggesting that the post-prandial hypercalcaemia stimulates and is controlled by CT release. Cooper & Deftos (1970) showed that the secretion of CT was increased after the intragastric administration of calcium chloride and also suggested that a mild hypercalcaemia arising from the absorption of calcium stimulated CT release directly. In addition, Pento (1971) has described preliminary experiments in the pig in which peripheral levels of CT are increased by the feeding of a high calcium diet. More recently the stimulatory role of pancreozymin (Care, 1970; Care & Bruce, 1971), of pentagastrin (Cooper, Schwesinger, Mahgoub & Ontjes, 1971) and of gastrin (Care, Bates, Swaminathan & Ganguli, 1971) on CT secretion has been demonstrated.

The present experiments have yielded two additional results. First, that immediately upon the presentation of food to the sheep there is a significant increase in the release of CT from the thyroid gland. Secondly, that after this initial rise in release the output of CT is maintained at a level similar to that before feeding for at least 3 h after the presentation of food.

The initial stimulation of release involves the activation of a b-adrenergic system within the thyroid gland caused possibly by the excitement associated with an impending meal since it is a transient change and can be blocked by the intraarterial infusion of (±)-propranolol at a concentration which has been shown to inhibit the normal adrenaline-stimulated CT release from the thyroid (Bates, Phillippo & Lawrence, 1970; Phillippo et al. 1970). In addition, this dose of propranolol, when distributed throughout the body, is over $1 \times 10^6$ times less than the lowest systemic b-blocking dose used during feeding experiments in sheep by Webster & Hays (1968). It is possible that the consumption of the barley diet at the start of feeding is linked with the increased CT secretion. In that event, the mechanism of action would not seem to involve the absorption of any appreciable amount of calcium from the diet since our unpublished data indicate that none of the barley diet passes into the abomasum during the initial phases of feeding and, in addition, there is no increase in the peripheral plasma calcium concentration at the start of feeding.

Several of the parameters that are known to change during feeding were measured.
in order to examine the role they played in the alteration of CT secretion. Stacy (1969) found that the blood pH remained at a decreased level during the entire period of feeding. Our own observations within these experiments support this finding, and this would suggest that pH changes are not associated with the change in CT secretion, since the release of CT returned to the level present before feeding within a short period even though the plasma pH remained at a decreased level throughout feeding. The changes in plasma calcium level reported by Stacy (1969) also occur at a much later time than the increase in secretion, and would not appear to be directly related to it. It is possible that the amount of ionized calcium in plasma may change during feeding and that this may influence CT secretion. Our preliminary results however indicate that the change in the ultrafiltrable calcium fraction in plasma appears to be unrelated to changes in the CT secretion rate.

During feeding the circulating plasma volume of the sheep decreases by approximately 15–20% (Blair-West & Brook, 1969; Christopherson & Webster, 1972). The present experiments have shown that the output of CT remains unchanged during feeding apart from the initial transitory rise in secretion. Theoretically one would expect that a combination of an unchanged secretion rate and of a decreased plasma volume would culminate in an increased peripheral level of calcitonin after feeding. Pento (1971) has described such a change in the peripheral CT levels after feeding in the pig. The occurrence of such a change remains to be confirmed in the sheep and its possible effects remain to be elucidated.

The present experiments would support the conclusion of Gray & Munson (1969) in the rat and of Pento (1971) in the pig that feeding affects the release of CT. In the sheep, however, in contrast to these other findings, the alteration of the hormonal release is of a transitory nature and the release is unaffected during the ensuing period of feeding.

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REFERENCES


