The salivary proteins secreted from cannulated parotid and mandibular glands of cattle after pharmacological stimulation

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ABSTRACT

Four cows were fitted with unilateral temporary cannulae to both parotid and mandibular glands in an experiment designed to determine which salivary proteins were secreted by these glands.

Parasympathomimetic stimulation produced a large salivary flow from the parotid gland (50.8 ml/min) with a relatively low protein concentration. Mandibular flow rates were lower, but the protein concentration was high giving a total protein of 1.3 mg/min. Sympathomimetic stimulation, during a parasympathomimetic booster, reduced the flow rates from both glands but increased the total protein output 7-fold from the parotid and 8-fold from the mandibular gland. Band 4 was the major protein secreted by the parotid gland while bands 3, 7 and 8 were the major bands secreted by the mandibular gland.

Keywords Salivary glands; parotid; mandibular; saliva; proteins; flow rates; pharmacological stimulation

INTRODUCTION

Rapid progress has been made in breeding cows for high and low susceptibility to bloat (Cockrem *et al.*, 1983). Studies on the salivary proteins have indicated a correlation between bloat susceptibility and specific salivary proteins (McIntosh and Cockrem, 1977) and furthermore it has been shown using bit collections that the concentration of band 4 and 6 proteins were increased when cows were grazed on bloat-potent pasture (Cockrem and McIntosh, 1978). The possibility of determining the phenotype of an animal, with respect to bloat susceptibility, on the basis of a ratio of salivary proteins was discussed by McIntosh and Cockrem (1982).

The data presented in this paper are from an experiment on 4 animals fitted with temporary parotid and mandibular gland cannulae, to ascertain which glands secrete the main low molecular weight proteins and to define the stimuli for their secretion.

EXPERIMENTAL DESIGN

Two high and 2 low susceptible cows were chosen on the basis of being extremes in susceptibility to bloat. These animals were in a state of good health, but with one high susceptible animal the cannulae were dislodged during collection of sympathomimetically stimulated secretion and data for this stimulation were therefore only collected from 3 animals.

Cows were tranquilised by intra muscular injection of Rompun (at a dose of 0.066 mg/kg), and propped in an upright lying position by hay bales. With the mouth of the animal held open by a metal gag, a cannula was inserted *via* the parotid papilla into the parotid salivary duct on one side of the mouth. A sample of the background flow of saliva was collected for a 3-minute period. It was not possible to locate the opening of the mandibular duct until after parasympathomimetic stimulation had induced saliva flow. This stimulus using carbachol was administered by intra muscular injection at a dose of 3 ml. Unilateral cannulation of the duct of a mandibular gland was possible once secretion flowed from its papilla. Three, 3-minute collections were made from both the parotid and mandibular ducts. Sympathetic stimulation utilised the sympathomimetic agent isoprenaline at a dose of 0.06 μg/kg/min and infused for 10 min *via* a venous cannula using a constant infusion pump. A carbachol booster was given (as above) after 10 min to prevent blockage of the mandibular cannula.

MATERIALS AND METHODS

Carbachol at a concentration of 1 mg/ml was supplied by May and Baker Ltd, Dagenham, England and isoprenaline sulphate by Burroughs Welcome and Co. Ltd, Rompun as a 2% solution for injection was supplied by Bayer, West Germany.

The diameter of the parotid cannulation tubing was I.D. 2.0 mm O.D. 3.0 mm and that for the mandibular duct was I.D. 0.75 mm O.D. 1.5 mm. The

infusion pump used in this experiment was made by Harvard Apparatus Co, Mass. USA.

Protein concentrations were determined by the method of Lowry *et al.* (1951) and the electrophoresis was performed according to the method of McIntosh *et al.* (1983). Corrected integration units were expressed for a standard 25 μl volume of saliva applied to the polyacrylamide gel. The total corrected integration units per minute (CIU/min) for a given protein band region were obtained by multiplying the CIU value by the saliva flow rate.

RESULTS

The average flow rate and total protein produced per minute from the cannulated parotid and mandibular glands under 3 different treatments are shown in Table 1.

TABLE 1 Average flow rate and total protein secretion for the parotid and mandibular glands during unstimulated (background), parasympathomimetic and sympathomimetic stimulation

	Flow rate ml/min	Total Protein mg/min
Parotid gland		
Unstimulated (background)	5.2	0.4
Parasympathomimetic	50.8 ± 3.7	1.2 ± 0.3
Sympathomimetic	30.8 ± 3.1	8.7 ± 1.7
Mandibular gland		
Parasympathomimetic	4.8 ± 0.7	1.3 ± 0.1
Sympathomimetic	3.4 ± 0.6	10.5 ± 2.5

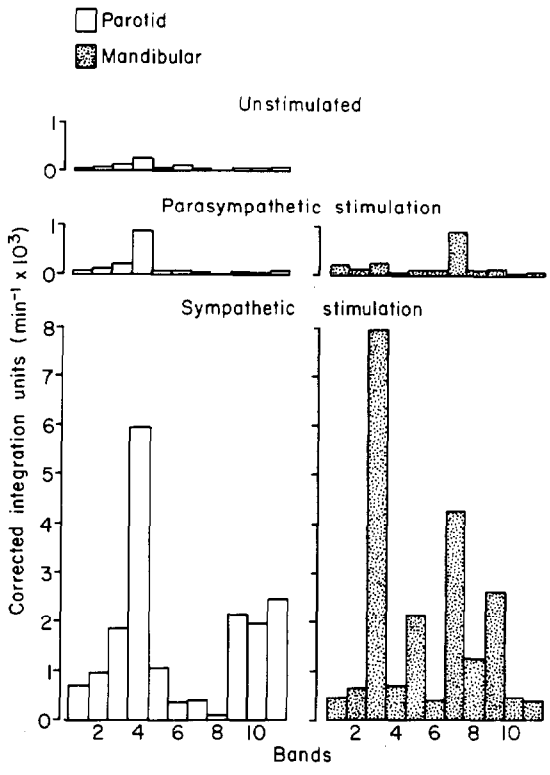

FIG. 1 Mean values for 3 animals for the major salivary proteins (CIU/min) secreted from the parotid and mandibular glands under 3 treatments, unstimulated (background), parasympathomimetic, and sympathomimetic stimulation.

Parotid

The background flow rate was 5.2 ml/min with a protein content of 0.4 mg/min. With parasympathomimetic stimulation there was a 10-fold increase in flow rate and a 2 to 3-fold increase in protein, and hence a decrease in protein concentration. The major protein produced was band 4 (Fig. 1).

Sympathomimetic stimulation with isoprenaline, during a parasympathomimetic booster, gave a 6-fold higher flow rate compared with the background value. The total protein secretion per minute was 7-fold greater than that produced by only parasympathomimetic stimulation. Band 4 protein was clearly in the highest concentration, but other proteins were present in intermediate concentrations and these were (in order of descending quantities) bands 11, 9, 10 and 3 (Fig. 1).

Mandibular

There was no collection from the mandibular glands prior to pharmacological stimulation, but carbachol produced an average flow rate of 4.8 ml/min and a protein content of 1.3 mg/min. The major protein entering the polyacrylamide gel was band 7 (Fig. 1).

Isoprenaline stimulation given in conjucntion with a parasympathomimetic booster, caused a small reduction in the flow rate, but produced an 8-fold increase in protein secretion. Band 3 protein was present in the highest concentration, followed by bands, 7, 9, 5 and 8 in order of descending concentration (Fig. 1).

The quantity of band 6 protein was similar in both the mandibular and parotid secretions under sympathomimetic stimulation.

When the quantities of band 4 protein (expressed as mg/min) are compared for each of the 3 collection periods on 3 cows under sympathomimetic stimulation (Fig. 2), it is clear that most of this protein was produced by the parotid gland. Likewise when band 7 plus band 8 proteins are plotted in the same manner (Fig. 3), these data show that most of these proteins originated from the mandibular secretions.

DISCUSSION

The classification of salivary proteins used in this paper was first described by Reid *et al.* (1974) and McIntosh (1975) and in more detail by McIntosh *et al.* (1983). These previous publications have presented data on

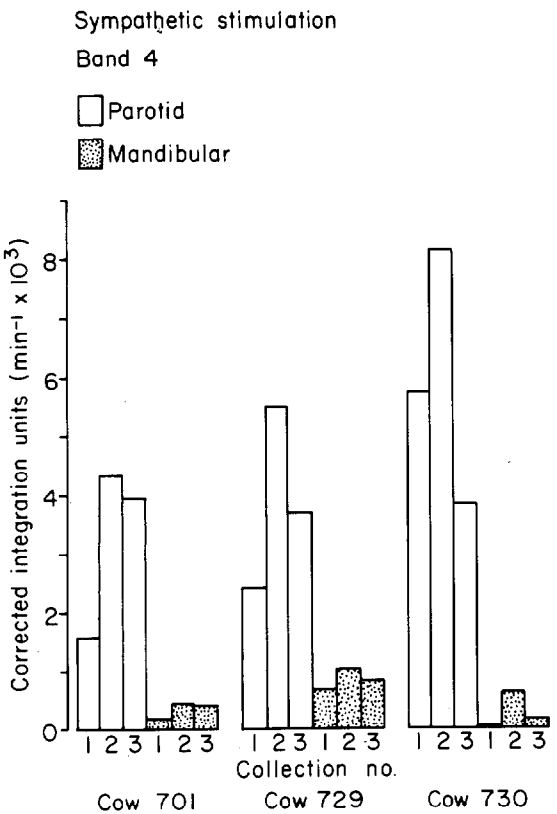

FIG. 2 The amount of band 4 (CIU/min) secreted from parotid and mandibular glands under sympathomimetic stimulation for 3, 3-minute collections from 3 animals.

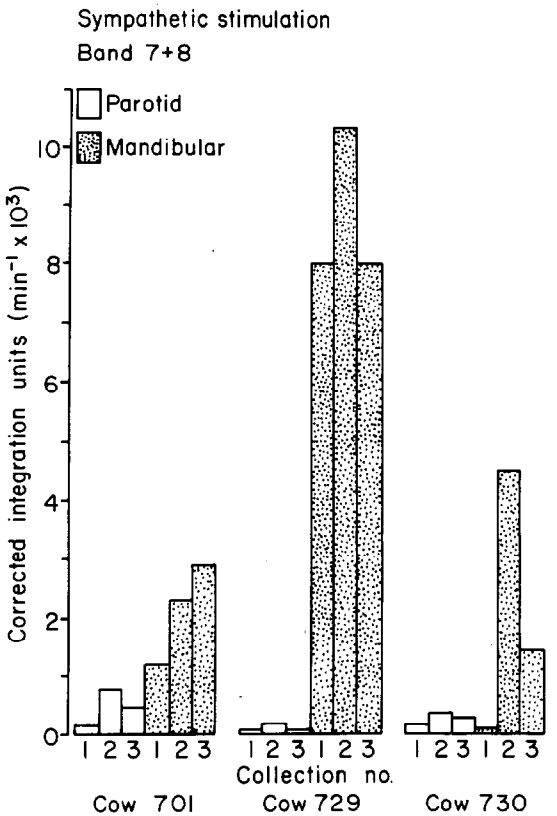

FIG. 3 The amount of bands 7 and 8 (CIU/min) secreted from parotid and mandibular glands under sympathomimetic stimulation for 3, 3-minute collections from 3 animals.

the salivary proteins as either percentages or corrected integration units. However it was possible to present these data as CIU/min as secretions were collected by a temporary cannula, rather than a saliva collecting bit which probably biased the collection towards mandibular saliva and was open to much greater sources of variation in measured flow rates. It is possible that the volumes from the mandibular gland are understated because the smaller diameter tubing used for the mandibular duct may have resulted in a loss of secretions from this gland, and furthermore, in this experiment there was no means of determining the physiological effects of the analgesic/anaesthetic drug Rompun. In relation to bloat susceptibility the way individual protein bands are expressed could be important as animal variation in both flow rate and protein concentration will be present. However, the purpose of this experiment was to determine which proteins were secreted from the parotid and mandibular salivary glands, and therefore only a small number of animals was used.

The electrophoretic data presented in Fig. 1 indicate that under sympathomimetic/parasympathomimetic stimulation most of the recognised array of major salivary proteins (McIntosh *et al.*, 1983) are secreted by both parotid and mandibular glands, but there is a clear cut quantitative difference between them for specific proteins. Most of the band 4 protein was shown to be secreted from the parotid gland, but band 4 protein from the mandibular secretions showed immunochemical cross reactivity. Evidence for this, and similar immunochemical data will be published elsewhere (McLaren *et al.*). Band 11 protein was predominantly from the parotid gland while bands 9 and 10 were in similar amounts from both glands. These 2 proteins appear chemically related with each other and with bands 7 and 8. Bands 7 and 8 proteins are chemically related (McIntosh, 1978) and they are predominantly secreted by the mandibular gland. Band 3 is also predominantly secreted by this gland but is in an intermediate quantity in parotid secretions, however this protein region is clearly heterogeneous (McIntosh, 1978). Band 5 is also produced in a higher amount by the mandibular gland than the parotid, but unlike band 3 it is most probably a single protein.

These data provide the first detailed account of

which proteins are secreted from the parotid and mandibular glands of cattle and this has enabled a mouth bit to be designed which collects saliva secretions independently from the front and rear of the mouth. This bit has been used in 2 trials relating to feeding regimes and further work on pharmacological stimulation, based on this present study, will be carried out using this collecting device.

ACKNOWLEDGEMENTS

To Alan Jackson and other staff members at Number 4 Dairy, Ruakura Animal Research Station.

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