The presence of kallikrein-like enzymes in bovine saliva

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ABSTRACT

Kallikrein-like enzyme (KE) activity has been detected in bovine saliva from the front of the mouth and from the cannulated mandibular gland. The maximal activity (39 mUnits ml^{-1}) was present in secretions from the mandibular gland of cows stimulated with carbachol (parasympathomimetic). A lower activity (24 mUnits ml^{-1}) was obtained under isoprenaline (sympathomimetic) combined with carbachol stimulation. No detectable KE activity was present in parotid gland secretions.

When tested for vasodilator activity in sheep, all samples tested were positive, but the greatest response was from mandibular secretions collected under carbachol stimulation.

Using purified salivary proteins, it was demonstrated that the KE activity was associated with bands 7 and 8, major proteins secreted by the mandibular gland.

Keywords Bovine saliva; kallikrein-like enzymes; parotid secretions; mandibular secretions; bloat.

INTRODUCTION

Kallikreins are enzymes which act on a specific protein or proteins (kininogens), cleaving off physiologically active peptides (kinins) which relax vascular smooth muscle and thus lower blood pressure and increase tissue permeability (Ganong, 1979).

Kallikrein-like enzymes (KE) have been reported in the salivary glands of man, guinea pig and dog (Matsuda *et al.*, 1979), rat (Brandtzaeg *et al.*, 1976), and mouse (Ekfors and Hopsu-Havu, 1971; Simson *et al.*, 1978), while McIntosh (1978) reported KE type activity in bovine salivary gland secretions.

In this paper further evidence is given for KE activity and its association with the protein bands suspected to be related to the susceptibility of cattle to bloat (McIntosh and Cockrem, 1982).

MATERIALS AND METHODS

Sampling

Two lactating cows of low and 1 of high susceptibility to bloat were brought into the yard after afternoon milking and left overnight with access to water only. After morning milking, 3, 3-minute saliva samples were collected from each using a 2-site bit (see below) after parasympathomimetic (carbachol) stimulation. This was followed by 3 similar collections after sympathomimetic (isoprenaline) stimulation, combined with the parasympathomimetic booster as described by McIntosh *et al.* (1984). The next day, after being kept in the yard overnight as described above, 1 high and 1 low cow were tranquilised by intramuscular injection of Rompun and temporary parotid and mandibular cannulae were inserted into each animal and secretions were collected as described by McIntosh *et al.*, (1984). Samples were collected at the same time intervals and under the same stimulation as used for the bit collections.

The stainless steel 2-site collecting bit (McIntosh *et al.*, 1984) collected separate saliva samples from close to the opening of the mandibular gland ducts (fore bit collection) and from close to the opening of the parotid ducts (rear bit collection).

Kallikrein Esterase Activity

This was measured by the method of Trautschold and Werle (1965) using N-α-Benzoyl arginine ethyl ester. Enzyme activity was expressed as mUnits ml^{-1} (mU) and calculated on the $\Delta A\ min^{-1}$ at a wavelength of 253 nm. All other biochemical measurements were performed as described by McIntosh *et al.* (1984).

Physiological Measurements of Vasodilators in Saliva

Experiments to assess vasodilator actions of the salivary proteins were performed under pentobarbitone sodium anaesthesia on Romney cross sheep of 35 to 45 kg body weight. Arterial blood pressure was recorded on a Gould chart recorder using Bell & Howell pressure transducers in a femoral artery. Salivary protein samples were injected into a femoral vein.

RESULTS

Mandibular Gland

With both the cannulated samples and those from the fore bit collection bands 7 and 8 were the main proteins secreted. The total concentration of protein

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was greater with isoprenaline but relative proportions of the 2 bands were similar for both types of stimulation (Table 1).

Parasympathomimetic treatment gave saliva with higher KE activity than the sympathetic for both methods of collection. The saliva from bit collections had lower activities than that from cannula collections (Table 1).

TABLE 1 Protein bands (%) for mandibular and fore bit saliva collections under parasympathomimetic (P), or parasympathomimetic combined with sympathomimetic (S) stimulation. Enzyme activity is expressed as mUnits ml^{-1} and total protein as $\mu g\ ml^{-1}$.

Band number	Cannulated		Bit	
	P	S	P	S
9	4	9	6	9
7, 7A, 8	36	34	33	32
7+8 only	31	30	31	30
5	11	9	6	7
4	7	6	9	5
3	12	20	17	15
Enzymic activity	39	24	16	8
Total protein	238	2259	444	2534

Parotid Gland

Cannulation samples had only 1/6th the total protein compared with mandibular secretions under sympathomimetic stimulation and band 4 was the major protein (23%). Parasympathomimetic stimulation gave too little protein for the bands to be measured.

The saliva from the rear of the bit showed 7 and 8 as the major bands with band 4 the next highest being 16% under sympathomimetic stimulation (Table 2).

KE activity for all samples was low or nil.

TABLE 2 Protein bands (%) for parotid and rear bit saliva collections under parasympathomimetic (P), or parasympathomimetic combined with sympathomimetic (S) stimulation. Enzyme activity is expressed as mUnits ml^{-1} and total protein as $\mu g\ ml^{-1}$.

Band number	Cannulated		Bit	
	P	S	P	S
9	-	12	7	11
7, 7A, 8	-	13	33	32
7+8 only	-	11	31	30
5	-	8	6	5
4	-	23	11	16
3	-	13	9	11
Enzymic activity	0	0	4	1
Total protein	21	337	259	678

Purified Protein Bands

KE activity was measured for a number of purified salivary proteins. Proteins purified from bands 7 and 7A region had the maximal activity (7 mU). Preparations containing some band 8 showed activity (2 mU), but purified proteins from bands 1, 2, 3 and 4 showed no activity.

Inhibitors

The esterase activity in saliva, and in a standard kallikrein preparation was not inhibited by soybean trypsin inhibitor. However aprotinin, a specific kallikrein inhibitor, inhibited the esterase activity of both the standard kallikrein and the saliva samples tested.

Vasodilators in Saliva

All samples tested possessed vasodilator activity but the greatest responses followed intravenous injection of samples derived from mandibular saliva. Fig. 1 shows the response to intravenous injection of mandibular salivary protein (0.15 mg/kg body weight). In this and in all other responses vasodilator activity appeared after a latency of about 20 sec and in the absence of gross changes in heart rate. Falls in arterial blood pressure ranged from 14 to 50% depending on the amount of protein injected (up to 0.3 mg/kg) and the conditions under which it was obtained.

In general, injection of equivalent amounts of protein produced greater responses when the sample had been secreted during parasympathomimetic stimulation.

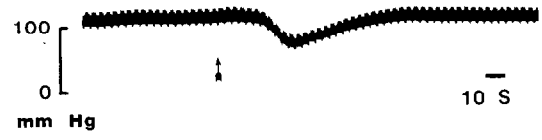

FIG. 1 Fall in arterial blood pressure following intravenous injection (arrow) of mandibular salivary protein obtained during parasympathomimetic stimulation.

DISCUSSION

The present results confirm those of McIntosh (1978) and also show that the KE activity (Trautschold and Werle, 1965) parallels vasodilator activity in the samples of various origins. This esterase activity was further confirmed as probable KE by its inhibition by aprotinin from bovine lung but not by soybean trypsin inhibitor.

The results with the purified proteins indicate that this activity was associated with the low molecular weight proteins, bands 7 and 8. In addition

the activity was greatest with parasympathomimetic stimulation, which had less total protein than sympathomimetic stimulated samples, suggesting the presence of an inhibitor produced by the sympathetic action. This could be associated with the greater amount of mucoprotein which binds other proteins or in the case of samples containing parotid saliva, with a specific KE inhibitor similar to, or the same as aprotinin which is known to be produced by the bovine parotid gland (Schachter, 1980).

The importance of these findings in relation to bloat susceptibility in cattle is still at an early stage of investigation. However, band 7 has been shown to be positively correlated with saliva flow as measured by the bit collections (McIntosh and Cockrem, 1977) and negatively related to band 4 protein. Band 4 protein is increased when cows are grazed on bloat-potent pasture (Cockrem and McIntosh, 1978), and furthermore the ratio of band 4 to bands 7 plus 8 gives an indication of the susceptibility status of animals (McIntosh and Cockrem, 1982). Schachter (1980) points out that contrary to previous ideas that salivary kallikreins were secreted into the blood circulation, it is now believed that the majority of the kallikrein is secreted in the saliva. Thus if the appropriate substrate is present (a kininogen), then kinins could be released and these peptide hormones could exert their physiological action on the vasculature of the papillae in the rumen by altering the blood pressure and tissue permeability. This could lead to the differences in rumen levels observed between cows selected for high or low susceptibility to bloat (Cockrem *et al.*, 1983). Finally, if band 4 protein from the parotid gland (Jones *et al.*, 1982; McIntosh *et al.*, 1984), was a kininogen then the observed relationship of the ratio band 4/(bands 7+8) to bloat susceptibility could well have biological validity.

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