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LATERAL NASAL GLAND SECRETION IN THE ANAESTHETIZED DOG

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SUMMARY

1. The effects of pharmacological and nervous stimuli on the flow of secretion from the dog lateral nasal gland following catheterization are described. Drugs were injected close-arterially into the arterial supply to the nose, or intravenously.

2. Cholinergic agonists (pilocarpine, methacholine), given intravenously (I.V.) or intra-arterially (I.A.), and stimulation of the vidian nerve produced a copious flow of secretion which was blocked by atropine. The adrenoceptor agonists phenylephrine (α) and salbutamol (β_2), given I.V. or I.A., and stimulation of the vagosympathetic nerve produced a small but consistent flow of secretion.

3. Histamine (50 μg), substance P (0·1 μg) and prostaglandin E_1 (1–5 μg), injected I.A., produced small flows of secretion. Bradykinin (25 ng–50 μg), 5-hydroxytryptamine (100 ng–50 μg) and vasoactive intestinal peptide (VIP) (10 ng–50 μg) did not cause secretion.

4. The total protein content, the composition of secretions as revealed by sodium dodecyl sulphate–polyacrylamide agarose gel electrophoresis, and changes in [Na] and [K] in relation to flow of secretion are described.

5. Differences in ion and protein concentrations, and in protein composition, are described for vidian nerve-induced and vagosympathetically induced secretions.

6. Electron microscopy revealed that the gland contains serous cells in the secretory region, and ducts morphologically similar to the intercalated, striated and excretory ducts of salivary glands.

INTRODUCTION

The lateral nasal gland of the dog is similar morphologically to a salivary gland. It is the phylogenetic equivalent of Steno's gland in the rat and the anterior nasal glands of many species including man. It lies on the wall of the maxillary sinus and possesses a long duct which opens into the vestibule of the nose (Broman, 1921; Bojsen-Moller, 1964, 1965, 1967; Moe & Bojsen-Moller, 1971; Klaassen, Van Wichen & Kuijpers, 1981; Kuijpers, Klaassen, Jap & Tonnaer, 1983). Several studies have indicated that secretions from the gland may have an important thermoregulatory function, particularly in animals which utilize thermal panting (e.g. pigs and dogs) rather than sweating (Schmidt-Nielsen, Bretz & Taylor, 1970; Blatt, Taylor & Habel, 1972). The rate of secretion increases markedly with increasing ambient air

temperature in conscious dogs (Blatt *et al.* 1972; Krausz, 1977), and the secretion is hypo-osmolar compared with tissue fluids.

Adams, De Young & Griffith (1981) have demonstrated a small resting secretion in conscious, chronically cannulated dogs. We have investigated the physiological and pharmacological factors controlling secretion from the gland in anaesthetized dogs, including stimulation of the vidian and vagosympathetic nerves and close intra-arterial injection of a variety of cholinergic and adrenoceptor agonists and mediators. Some biochemical and histological studies have also been done. An abstract of some of the results has been given (Nathanson, Wells & Widdicombe, 1985).

METHODS

Dog experimental procedure

Adult greyhounds of either sex, 20–32 kg, were anaesthetized with sodium thiopentone (Intraval, May & Baker Ltd., 30–35 mg kg^{-1}) followed by either chloralose (α-glucose chloralose, B.D.H. Chemicals Ltd., 80–100 mg kg^{-1}) or sodium pentobarbitone (Sagatal, May & Baker Ltd., 36 mg kg^{-1}) into a leg vein.

The dog was initially tied down supine. The lower cervical trachea was cannulated to allow breathing. A femoral artery and vein were catheterized using polyethylene catheters (Portex). Blood pressure was monitored from the femoral artery using a Statham pressure transducer. Tidal volume and breathing frequency were measured using a Fleisch pneumotachograph attached to the tracheal cannula and electrical integration. When required, further doses of anaesthetic were administered via the femoral vein. Rectal temperature was monitored throughout.

The dog was then turned prone. The zygomatic arch on one or both sides of the head was removed and the underlying nerves were exposed. The terminal branch of the maxillary artery was catheterized retrogradely using a polyethylene catheter (internal diameter 0·75 mm, Portex). A snare was placed around the maxillary artery proximal to the sphenopalatine artery. This allowed close intra-arterial injection of drugs to the sphenopalatine artery which supplies 75 % of the walls of the nasal cavity, including the lateral nasal gland; in some experiments there was separate perfusion of the sphenopalatine artery (Lung, Phipps, Wang & Widdicombe, 1984).

When required, the vidian nerve was isolated, dissected free of surrounding tissue, ligated and cut. The cut peripheral end of the nerve was stimulated electrically at 1–5 V, 10–80 Hz, 2 ms duration for 15–60 s. Maintained stimulation of the nerve was performed in three dogs. When required, the vagosympathetic nerve (united in the dog) was exposed in the neck below the superior cervical ganglion and ligated. The nerve was cut before electrical stimulation of the central (cranial) end at 20 V, 20 Hz, 2 ms for periods up to 10 min.

The dog was then maintained prone with the lower jaw supported. An incision was made in the dorsal wall of the nose just lateral to the septum for a distance of about 2 cm posterior to the tip, until the nasal bone was reached. The orifice of the duct of the lateral nasal gland was exposed on the lateral wall, about 2 cm posterior to the alae nares (Evans, 1977), and catheterized with polyethylene tubing (internal diameter 0·75 mm, Portex). The catheter was pushed 2 cm into the duct and then secured by a suture at the tip of the nose. In most experiments the ducts on both sides of the nose were catheterized.

Samples were collected by placing the free end of the gland catheter into a pre-weighed collection tube of volume 400 μl (Alpha Laboratories). The collection time and weight of the samples were recorded. Samples were stored frozen until required for further analysis.

The following drugs were used: pilocarpine hydrochloride (Sigma), acetylcholine chloride (Sigma), methacholine chloride (Sigma), atropine sulphate (Sigma), phenylephrine hydrochloride (Boots), salbutamol sulphate (Allen & Hanburys), dobutamine hydrochloride (Lilly), bradykinin triacetate (Sigma), 5-hydroxytryptamine hydrochloride (Sigma), histamine hydrochloride (Sigma), vasoactive intestinal peptide (VIP) and substance P (Cambridge Research Biochemicals), prostaglandins E_2 and $F_{2\alpha}$ (Sigma), propranolol hydrochloride (I.C.I.), phenoxybenzamine hydrochloride (S.K.F.).

Drugs were given as single injections either into the femoral vein (I.V.) or into the nasal artery (I.A.) and were diluted in 0·9 % heparinized saline. The effects on secretion are presented as mean

flow (μl min^{-1}) when flow was maintained for long periods, but in other cases as total weight of secretion (mg). The qualitative effects of stimuli on secretion were analysed using the sign test.

Histology

The glands were identified by retrograde injection of Indian ink via the catheter in the gland duct. They were exposed by removal of the cheekbone around the maxillary sinus and the lateral wall of the nose. One gland was fixed with 10% buffered formol saline by retrograde injection into the collecting duct and via a cannula in the carotid artery, after blood had been washed from the circulation with saline. Gland and duct tissue were then embedded in wax, sectioned and stained either with haematoxylin and eosin, Periodic Acid–Schiff's (PAS) reagent and/or Alcian Blue at pH 2·6.

Two glands required for electron microscopy were fixed using 5% glutaraldehyde at 5 °C, given as with formol saline. Tissues were secondarily fixed with 1% osmium tetroxide and embedded in Araldite CY212 resin before sectioning. One of these glands had not been stimulated, whilst the other had been subjected to a series of short vagosympathetic nerve stimulations and to a maintained vidian nerve stimulation.

Analysis of secretions

The total protein content of samples was estimated by the Lowry method (Lowry, Rosebrough, Farr & Randall, 1951). Sialic acid content was measured using the thiobarbituric acid assay (Warren, 1959). Lysozome activity was measured by the method of Lorenz, Korst, Simpson & Musser (1957) in arbitrary units and converted to μg ml^{-1} by comparison with egg white lysozyme chloride standards. The concentrations of Na and K were obtained using either a Corning–Eel flame photometer or a Pye Unicam SP9 atomic absorption spectrophotometer. Chloride concentrations were obtained using a digital chloridometer (Buchler Instruments).

Sodium dodecyl sulphate–polyacrylamide agarose electrophoresis

Gels consisting of 2% acrylamide–0·5% agarose in 0·03 M phosphate buffer (containing 1 mM-EDTA and 0·1% sodium dodecyl sulphate (SDS), pH 7·0) were prepared using a combination of the methods of Peacock & Dingman (1968), Holden, Yim, Griggs & Weisbach (1971*a*, *b*) and Sachdev, Fox, Wen, Schroeder, Elkins & Carubelli (1978). The gels were cast in 12 cm gel tubes.

Secretions were reduced using 0·1 M-dithreitol/0·1% SDS in 0·1 M-Tris HCl buffer (Roberts, 1976). *S*-carboxymethylation was carried out using the method of Liao, Blumenfeld & Park (1979). The treated secretions, mixed with 10% glycerol and Bromophenol Blue, were added to each gel. Gels were run using an LKB Shandon disk electrophoresis apparatus (5 mA/tube, 55 mV, 2 h).

Gels were stained for protein using 0·03% Coomassie Blue R-250 in 25% propan-2-ol/10% acetic acid. Acidic glycoproteins were stained using 1% Alcian Blue in 7·5% acetic acid. A modification of the PAS method (Kapitany & Zebrowski, 1973) was used to stain neutral glycoproteins. Exhaustive destaining of gels using 7·5% acetic acid was continued for 2–3 days.

Mobilities of bands on each gel were recorded and the approximate molecular weights of each band assessed by comparison with the results from the marker gel containing bands of known molecular weight in the range 50000–336000. Over this range the relationship between logarithm of molecular weight mobility was linear. The assumption was made that this relationship also applied to bands of molecular weight outside this range.

RESULTS

Factors stimulating secretion

The right vidian nerve was stimulated sixteen times at various frequencies and strengths in the absence of blocking agents, and a further fifteen times in the presence of various combinations of blocking drugs, in a total of seven dogs. The mean weights of secretions obtained are shown in Table 1. Resting control secretion rates in the absence of nerve or drug stimulation were always too small to be measured.

Nerve stimulation in the absence of blocking agents resulted in a rapid and copious

flow of secretion. Secretion was first observed in the catheter in the collecting duct about 30 s after the beginning of stimulation. Secretions from the contralateral gland were absent or too small to measure.

The effects on secretion were considerably reduced by atropine sulphate (0·25–1·00 mg kg^{-1}, I.V.). Whether adrenoceptor antagonists affect the secretory response is unclear from the results, although the presence of phenoxybenzamine (0·5–1·0 mg kg^{-1}, I.V.) and propranolol (0·5 mg kg^{-1}, I.V.) appeared to reduce secretion further after atropine administration. The effects of these blocking agents on vidian nerve stimulation before atropinization were not tested.

TABLE 1. Flow of secretions in response to vidian nerve stimulation

Stimulation duration (s)	Antagonists	n	Wt. of secretion: Mean (mg)	Wt. of secretion: S.E. of mean or range (mg)	Protein concentration (mg ml^{-1})
15	None	2	114	104–124	2·6, 2·1 (2)
30	None	11	231	±26**	2·1±0·45 (9)
60	None	3	143	111–162	1·7, 3·0 (2)
30	Atr.	4	24	1–62	1·5 (1)
60	Atr.	4	23	0–60	
30	Atr., PB	2	18	9–27	4·3 (1)
30	Atr., PB, Prop.	5	4	±5	

Frequencies were all 20 hz and intensities 1–3 V. Atr = atropine; PB = phenoxybenzamine; Prop. = propranolol. n values for proteins are in parentheses. ** $P < 0·01$.

Unilateral vagosympathetic nerve stimulation for periods of 30 s–2 min in five dogs produced a mean weight of secretion from the ipsilateral gland of 30±11 mg ($n = 20$). The lack of a clear relationship between stimulation duration and response may indicate that the responses were maximal. In the presence of atropine, the mean weight of secretion was 2±2 mg ($n = 4$) (Table 2). Occasionally very small flows occurred on the contralateral side (mean 1±0·2 mg, $n = 13$).

In a further thirteen 3 min nerve stimulations in three dogs, the mean output was 24±15 mg on the ipsilateral side and 9±6 mg on the contralateral side.

In view of the fact that stimulation of the central end of the vagus caused apnoea (by the Breuer–Hering reflex), we induced apnoea by occluding the tracheal tube for 30 s. This never caused secretion.

Pilocarpine (0·25 mg kg^{-1}, I.V.) in three dogs produced a rapid secretory response; this was measured in two dogs. An initially high flow of secretion declined over a period of 90 min. The total weights of secretions over 90 min were 1·1 and 5·1 g respectively. Mucus dripped from both sides of the nasal cavity, and lacrimation and salivation also occurred. In another three dogs, pilocarpine (0·25 mg kg^{-1}, I.V.) was given after atropine (0·25 mg kg^{-1}, I.V.) but no secretion occurred.

Methacholine (100 μg, I.A. in seventeen tests) produced a large flow of secretion (330±150 mg over 3–5 min) on the ipsilateral side, and a smaller flow (57±32 mg) on the contralateral side (Table 3). Both responses were blocked by atropine I.V. ($n = 9$). In two other experiments, doses of 50 μg and 0·25 μg I.A. caused total ipsilateral secretions of 142 and 350 mg respectively, with no contralateral response.

Acetylcholine, in doses increasing 10-fold from 0·4 ng to 4 μg I.A. in one dog, caused dose-related secretions of 22–133 mg on the ipsilateral side.

Intra-arterial injections of phenylephrine (50–200 μg) and salbutamol (50–100 μg) consistently produced small flows of secretion, although far less than those produced by methacholine (Table 3). Surprisingly in three dogs the actions of phenylephrine ($n = 3$) and salbutamol ($n = 1$) were almost completely blocked by atropine I.V. The response to salbutamol was almost completely blocked by propranolol ($n = 3$). Dobutamine (50–100 μg) was given I.A. four times in three dogs. Ipsilateral secretion was 38 ± 27 mg. There were small flows of secretion on the contralateral side with all three catecholamines.

TABLE 2. Flow of secretions in response to cervical vagosympathetic nerve stimulations

Stimulation duration (s)	Antagonists	n	Wt. of secretions (mg) (mean ± S.E.)	Protein concentration (mg ml^{-1})
30	None	6	29 ± 17	11·3, 6·4, 4·3 (3)
60	None	10	27 ± 19*	8·1 (1)
120	None	4	39 ± 17	18·3, 5·6 (2)
30–120	Atropine	4	2 ± 2	

Frequencies were all 20 Hz and intensities 20 V. n values for proteins in parentheses. * $P < 0.05$.

TABLE 3. Flow of secretion in response to I.A. injections of agonists and mediators

Stimulus	Dose (μg)	N	Mean secretion (means ± S.E. of means) Ipsilateral (mg)	Contralateral (mg)	Protein concentration (mg ml^{-1})
Methacholine	100	17/17	330 ± 150**	57 ± 32	2·02 (1)
Phenylephrine	50–200	12/9	59 ± 46**	17 ± 24	4·77, 10·6, 12·7 (3)
Salbutamol	50–100	13/8	17 ± 9**	2 ± 3	2·45, 11·4 (2)
Dobutamine	50–100	4/4	38 ± 27	6 ± 6	3·9, 4·3 (2)
Histamine	50	9/9	59 ± 31**	5 ± 8	3·1 (1)
Substance P	0·1	8/7	59 ± 80**	3 ± 1	—
PGE_1	1–5	14/11	28 ± 15**	0	—

N = Number of tests/number of positive responses. n values for proteins are in parentheses. ** $P < 0.01$.

A variety of mediators were tested. Histamine (50 μg), substance P (0·1 μg) and prostaglandin E_1 (PGE_1) (1–5 μg) consistently produced small flows of secretion (Table 3). Substance P (10 ng, $n = 5$), bradykinin (25 ng–50 μg, $n = 7$), 5 HT (100 ng–50 μg, $n = 7$) and VIP (10 ng–50 μg, $n = 8$) did not induce secretion. These substances were then given 2 min after administration of 100 μg methacholine (VIP: 1 μg, $n = 9$; bradykinin: 1 μg, $n = 1$; 5 HT: 1 μg, $n = 1$) to test whether they might augment a cholinergically induced flow of secretion. No such effect was observed. All these doses of drugs produced large nasal vascular changes in the same dog preparations (see Discussion).

Maintained stimulation of the vidian nerve (3 V, 20 Hz, 2 ms) for a period of

40–60 min was performed in three dogs. Changes in the secretory pattern in one dog are shown in Fig. 1; a similar pattern was obtained on each occasion. In each the initially high rate of secretion (mg min^{-1}) declined rapidly: 264 ± 183 after 1 min; 195 ± 30 after 2 min; 190 ± 45 after 3 min; 177 ± 72 after 4 min; 126 ± 84 after 5 min ($n = 3$). A steady state of secretion was reached after 10–12 min. In one animal, atropine (0·5 mg kg^{-1}, I.V.), given during maintained stimulation, abolished secretion completely.

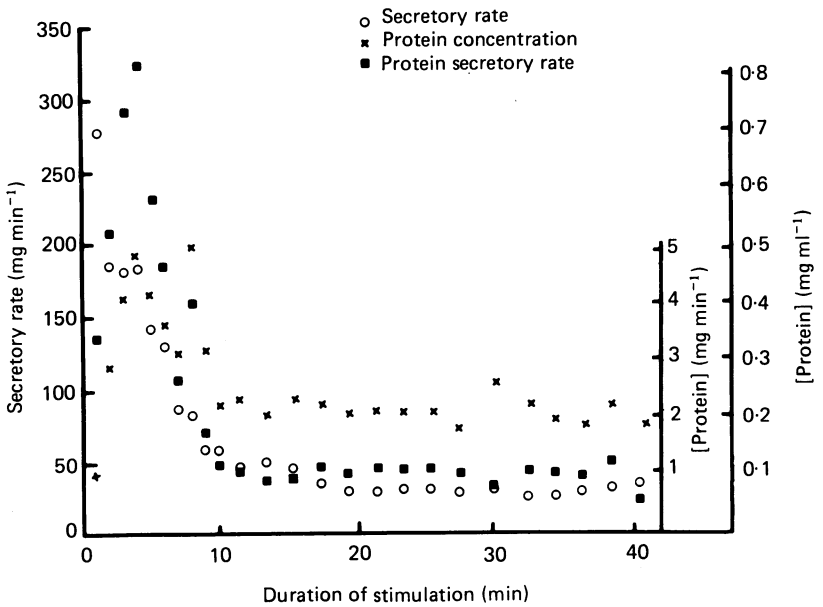

Fig. 1. Graph showing the secretory rate (mg min^{-1}), protein secretory rate (mg min^{-1}), and protein concentration (mg ml^{-1}) of lateral nasal gland secretions collected during 42 min stimulation of the vidian nerve (3 V, 20 Hz, 2 ms).

Similar results were obtained by I.V. injections of pilocarpine (see above) and by I.A. infusion of methacholine (10–100 μg min^{-1}). All results show that, after an initial large secretion, the gland is capable of maintaining secretions of over 25 mg min^{-1} for more than 1 h.

Macromolecular composition

The secretion of the dog lateral nasal gland contains proteins, including albumen and IgA, but more detailed analysis does not seem to have been carried out (Adams *et al.* 1981). We have measured the total protein and sialic acid concentrations and lysozome activity. SDS–polyacrylamide agarose electrophoresis revealed further information about the composition of the secretions. Some protein concentrations are given in Tables 1–3.

The change in protein concentration (mg ml^{-1}) and protein secretory rate (mg min^{-1}) with time in secretions produced by maintained vidian nerve stimulation were measured in three dogs. The results of one are shown in Fig. 1. The others

produced similar patterns. An initial increase in protein secretory rate reached a peak at 4 min after the onset of stimulation and declined thereafter. The decline in the total output of protein mirrors the decline in flow and reaches a plateau after about 12 min. Similar results were obtained with infusions of pilocarpine I.V. and methacholine I.A..

The mean protein concentration in samples produced by brief stimulation of the vidian nerve (1–2 V, 20 Hz, 2 ms, 15–120 s, $n = 13$) was $2{\cdot}2 \pm 0{\cdot}38$ mg ml^{-1}, whilst in samples obtained by vagosympathetic nerve stimulation (20 V, 20 Hz, 2 ms, 30–120 s, $n = 6$) it was $9{\cdot}0 \pm 3{\cdot}7$ mg ml^{-1}.

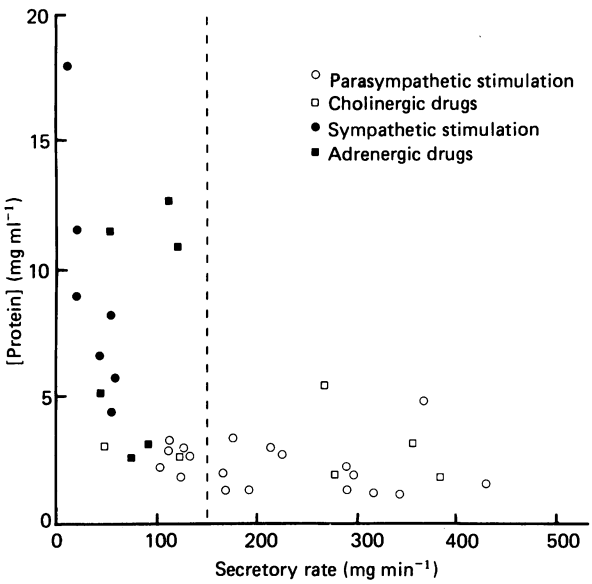

Fig. 2. Relationship between protein concentration (mg ml^{-1}) and secretory rate (mg min^{-1}) for parasympathetic (vidian nerve) stimulation (○), close-arterial injection of cholinergic drugs (□), vagosympathetic nerve stimulation (●), and adrenergic drugs (■). For further description see text.

The protein concentrations of secretions produced by a variety of stimuli are shown in Fig. 2. At high flows (above about 150 mg min^{-1}) protein concentrations were usually below 3 mg ml^{-1}. Such high flows, however, were only induced by cholinergic agonists or vidian nerve stimulation. There was no relationship between these flow rates and protein concentration (open symbols).

At lower flows a wide range of protein concentrations (2·5–18 mg ml^{-1}) were obtained. Low flows were produced mainly by vagosympathetic nerve stimulation, adrenoceptor agonists and mediators; comparison with protein concentrations at similar low flows induced by vidian nerve stimulation and cholinergic agents indicates that the latter cause a lower protein concentration (points to left of dashed vertical line in Fig. 2) ($P < 0{\cdot}05$).

The sialic acid concentration was measured in twelve samples obtained by vidian nerve stimulation (1–3 V, 20 Hz, 2 ms) for periods of up to 2 min in one dog. The mean

concentration was $73 \pm 12{\cdot}8$ μg ml^{-1}, compared with 82 and 124 μg ml^{-1} in whole nasal mucus.

Lysozyme activity was investigated in twenty-eight samples of nasal gland secretion. These were produced by a variety of stimuli, and there was no clear distinction between the level of activity and the stimulus type. The mean value was 61 ± 14 u. ml^{-1}, which corresponds to $2{\cdot}7 \pm 0{\cdot}62$ μg ml^{-1}.

With SDS polyacrylamide agarose gel electrophoresis, secretion collected after 6 min of maintained vidian nerve stimulation (3 V, 20 Hz, 2 ms) gave a total of six bands. The approximate molecular weights were 51000, 87000, 116000, 275000, 380 000 and 1480000. All stained with PAS except the highest molecular weight band. All stained with Coomassie Blue except the 87000 molecular weight band. Secretion

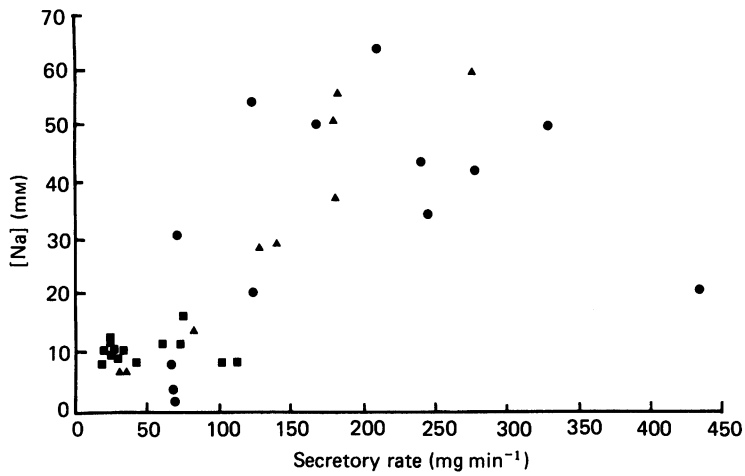

Fig. 3. Graph showing the relationship between [Na] (mM) in lateral nasal gland secretions and the secretory rate (mg min^{-1}). Samples were obtained by maintained stimulation of the vidian nerve in three dogs (different symbols).

collected after 17–20 min of maintained vidian nerve stimulation contained four bands of molecular weight 51000, 275 000, 380000 and 1350 000. All four bands stained with Coomassie Blue, but only the 380000 molecular weight band stained with PAS or Alcian Blue. Samples obtained by stimulation of the vagosympathetic nerve contained three bands of molecular weight 51000, 380000 and 1010000, all of which stained with Alcian Blue. The highest molecular weight band did not stain with Coomassie Blue. The lowest molecular weight represented the average molecular weight of a very broad band and presumably contained various proteins of low molecular weight.

Ionic concentrations of secretions

The [Na] produced by maintained vidian nerve stimulation in three dogs are plotted against flow rate in Fig. 3. The [Na] rose with increasing flow rate. This strongly suggests that reabsorption of Na occurs in the collecting ducts.

The [K] of the same secretions are shown in Fig. 4. Although [K] tended to be lower at high flow rates, the relationship for individual experiments was less clear.

Chloride concentrations were in the range 11–107 mM, with higher concentrations at faster flow rates.

Stimulation of the vagosympathetic nerve caused small secretions below 60 mg min^{-1}, as already described. The mean ion concentrations of these secretions were $25{\cdot}3 \pm 5{\cdot}2$ mM for Na ($n = 9$) and $64{\cdot}9 \pm 8{\cdot}13$ mM for K ($n = 7$). For rates of secretion below 50 mg min^{-1} (Figs. 3 and 4), vidian nerve stimulation produced secretion with [Na] of $9{\cdot}82 \pm 0{\cdot}83$ mM ($n = 11$) and with [K] of $32{\cdot}2 \pm 3{\cdot}33$ mM ($n = 11$). Both ion concentrations were significantly ($P < 0{\cdot}01$) lower for vidian nerve compared with vagosympathetic nerve stimulation.

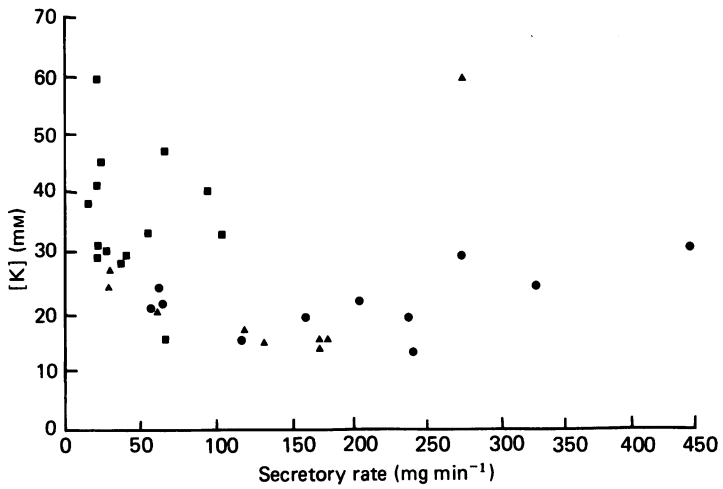

Fig. 4. Graph showing the relationship between [K] (mM) in lateral nasal gland secretions and the secretory rate (mg min^{-1}). Samples were obtained by maintained stimulation of the vidian nerve in three dogs (different symbols).

Histology

Glands were dissecting after retrograde injection of Indian ink into the collecting duct. The length of the ducts was 4–5 cm. The glands were about 3–4 cm long, 2 cm wide and 2–3 mm thick.

With light microscopy all acini and secretory cells stained with both Alcian Blue (pH 2·6) and PAS. When both stains were used together, the predominant colour was blue (although a pinkish tinge was apparent over some acini), suggesting that the glycoproteins were mainly acidic. Duct cells did not stain with Alcian Blue or PAS. In mucosal tissue surrounding the gland, submucosal glands stained pink primarily (neutral glycoprotein), whilst of the epithelial cells some were purple (mixture of acidic and neutral glycoproteins) and some blue (acidic glycoproteins).

Electron microscopy showed that acinar cells were pyramidal serous cells, about 10–20 μm in diameter, and contained large basal nuclei, prominent Golgi apparatus and extensive rough endoplasmic reticulum, particularly in the mid and basal regions of the cell. Numerous mitochondria were present basally. Cytoplasmic interdigitations and desmosomes were present on the lateral wall. Collagen fibrils were abundant

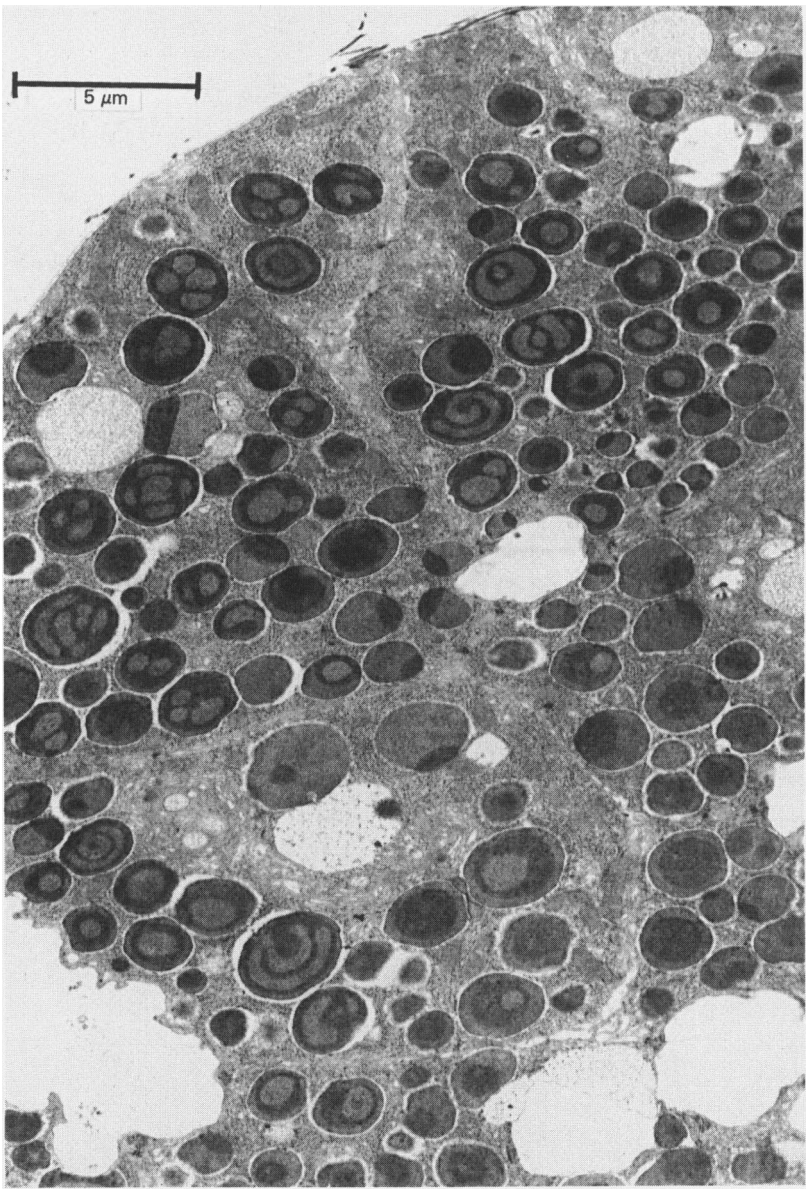

Fig. 5. Electron micrograph of acini from unstimulated lateral nasal gland. Glutaraldehyde and osmium tetroxide; uranyl acetate and lead citrate. Magnification × 4800.

around the edges of the acini. In the unstimulated gland numerous discrete electron-dense granules were distributed throughout the cell; diameters varied greatly in the range 0·5–2·5 μm. Three types of granule could be distinguished (Fig. 5): (1) large vesicles with a granular appearance, which might be pre-secretory granules (cf. those in Paneth cells of duodenum: Rhodin, 1974); these granules were rather rare; (2) granules consisting of two zones of different electron densities; (3) granules with a more complex distribution of electron densities; in the non-stimulated gland these granules were commonest (Fig. 5). In a gland which had been stimulated

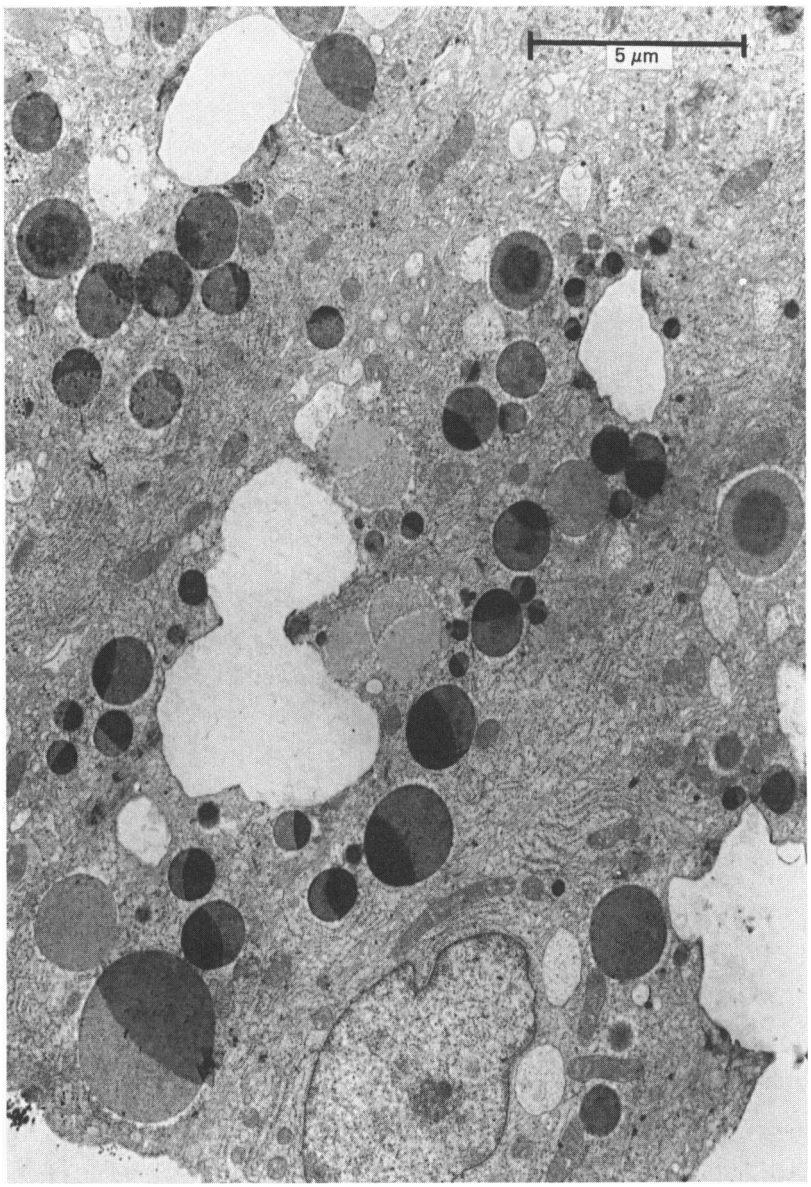

Fig. 6. Electron micrograph of acini from gland following prolonged stimulation of vidian and vagosympathetic nerves. Glutaraldehyde and osmium tetroxide; uranyl acetate and lead citrate. Magnification × 5400.

via the vagosympathetic and vidian nerves, there were fewer granules and none of the third type was present (Fig. 6). All nerve fibres seen near acini were non-myelinated. Most were located in connective tissue surrounding acini. Only one nerve fibre was observed between acinar cells.

Electron microscopy showed that the lateral nasal gland contains ducts morphologically similar to the intercalated, striated and excretory ducts of salivary glands.

DISCUSSION

In general no resting secretion was observed, in contrast to the resting flow of 0·20–0·40 ml h^{-1} reported in conscious dogs (Blatt *et al.* 1972; Adams *et al.* 1981). This difference is presumably an effect of anaesthesia and has also been reported in the major salivary glands (Jenkins, 1978). However, our sampling period (5–15 min) may have been too short to measure a small resting secretion.

The main control of secretion appears to be cholinergic, since cholinergic agonists and vidian nerve stimulation produced high flows which could be blocked by atropine. Similar flows also occur in salivary glands during maintained parasympathetic nerve stimulation (Terroux, Sekelj & Burgen, 1959; Schneyer, Sucanthapree & Schneyer, 1977) and with cholinergic agonist *in vivo* (Coroneo, Dennis & Young, 1979) or *in vitro* (Case, Conigrave & Young, 1977; Case, Conigrave, Novak & Young, 1980; Compton, Martinez, Martinez & Young, 1981). The decline in flow rate is probably due to exhaustion. It should be noted that the vidian nerve also contains sympathetic nerves to the nose (Eccles, 1982).

Krausz (1977) reported that spontaneous flow of secretion by the lateral nasal gland of conscious dogs was *reduced* by acetylcholine and further diminished by atropine. It is difficult to account for the difference between his results and those in this study merely in terms of anaesthesia. The effect described by Krausz (1977) might perhaps be due to a large acetylcholine-induced drop in glandular perfusion pressure.

Stimulation of the vagosympathetic nerve and I.A. infusion of all three adrenoceptor agonists promoted a small flow of secretion, suggesting that α-, β_1- and β_2-adrenoceptors are present on nasal gland acinar cells. Similar effects have been noted in salivary glands (Langstroth, McRae & Stavraky, 1938; Schneyer, Young & Schneyer, 1972; Schneyer *et al.* 1977). These stimuli also increase mucus glycoprotein secretion in the rabbit nose (Pell, Phipps, Wells & Widdicombe, 1984). Malm, McCaffrey & Kern (1983) demonstrated that phenylephrine applied locally to the dog nose can augment a methacholine-induced secretion, and that this effect is blocked by phentolamine. However, their method does not distinguish between secretions from lateral nasal glands and other sources. We were surprised that, in a few experiments, atropine inhibited the secretory responses to vagosympathetic stimulation and catecholamines; they may have depended on a subthreshold level of cholinergic tone.

Substance P, PGE_1 and histamine also produced secretion at low flows. Substance P-like immunoreactivity has been demonstrated in neurones (probably sensory) of salivary glands and in the nasal and tracheal mucosa (Hokfelt, Johansson, Kellerth Ljungdahl, Nilsson, Nygards & Pernow, 1977; Lundblad, 1984). In the rat submandibular gland, substance P has a direct stimulatory action on acinar and duct cells, and also has a modulating effect on the action of neurotransmitters (Martinez & Martinez, 1981). Substance P stimulates mucoglycoprotein output from tracheal explants (Coles, Bhaskar, O'Sullivan & Reid, 1984). Little is known about prostaglandin action on the secretion of nasal mucus from sources other than the lateral nasal gland, although the effects on nasal vasculature have been described (Jackson, 1970; Lung *et al.* 1984). In the cat trachea a variety of prostaglandins increase mucin secretion (Richardson, Balfre & Hall, 1978). Histamine increases nasal secretion in

man (Melon, 1968; Konno & Togawa, 1979; Mygind, 1979) and the rat (Burns & Williams, 1977), but has little effect in the rabbit (Pell *et al.* 1984), and increases nasal vascular permeability in the dog (Jackson & Burson, 1977).

The lack of effect of VIP on secretion, even on a methacholine-induced flow, is of interest since it has been suggested that VIP may act as a modulator of acetylcholine-induced secretion as well as a cotransmitter in terms of its vasodilator action (Barnes, 1984). VIP immunoreactivity has been demonstrated in the nasal mucosa of a variety of animals (Uddman, Malm & Sundler, 1980), and has a similar distribution to cholinergic fibres. Neither bradykinin nor 5 HT had secretory effects, suggesting that their importance (if any) in the nose may be confined to their vascular actions.

We did not establish dose–response relationships for any drugs, but the intra-arterial doses were chosen as those adequate to cause large changes in vascular resistance and airflow resistance in the dog nose with nasal arterial perfusion (Lung *et al.* 1984); the same experiments showed a 10% cross-over of nasal circulation, that may explain some of the contralateral responses with drugs.

Secretory composition

Fig. 2 suggests that the protein concentration of gland secretions is determined not only by flow rate but also by the nature of the stimulus itself. Thus the higher concentrations in the vagosympathetic- and adrenoceptor-stimulated samples are not merely an effect of the smaller volumes of secretions. However, more experiments are required to establish this, and at low flow rates the protein concentration may be affected by previous interventions due to the 'dead space' of the ductal system. For saliva it is generally considered that the secretory rate and not the stimulus route determines protein concentration (Langstroth *et al.* 1938; Komarov & Stavraky, 1940; Yamamoto, Inoki, Tsumimoto & Kojima, 1968; Young, 1979). With lysozome activity we found no difference in concentration with different modes of stimulation.

The protein concentrations in vidian nerve-stimulated samples are similar to the 2·03 mg ml^{-1} in the resting secretions obtained by Adams *et al.* (1981) and contrast with the 0·1 mg 100 ml^{-1} in the thermally stimulated secretions reported by Blatt *et al.* (1972). The reason for this large difference is unknown.

The initial peak in protein secretory rate during maintained vidian nerve stimulation may represent the combination of the secretion of pre-formed mature granules and an increased synthetic rate in acinar cells. The plateau presumably represents the turnover of newly synthesized protein granules and depends on the synthetic rate. However, it is clear that the gland can produce copious flows over long periods, which is consistent with its postulated role in thermal control.

The ion concentrations are consistent with the behaviour of sweat and salivary glands, as is the histological appearance of the striated duct regions. The effects of flow rate on ion concentrations are similar to those found in salivary glands (Young, 1979), and at fast flow rates the secretion is very hypotonic, appropriate to its activity in hyperthermia (Blatt *et al.* 1972). Our results suggest that secretion induced by sympathetic nerves may have a higher [Na] and lower [K] than that induced by parasympathetic stimulation. Our method measured total [Na] and [K] (ionized plus un-ionized) and some cations would be bound to proteins. However, the difference between [Na] and [K] is unlikely to be explained by different protein contents, and

possibly the two innervations have different actions on the ion-exchange regions of the duct system.

SDS–polyacrylamide agarose gel electrophoresis showed a number of bands, the highest of which had a molecular weight typical of a mucoglycoprotein. With vagosympathetic nerve stimulation and with long-maintained vidian nerve stimulation, fewer bands were seen, especially at the lower molecular weights. This suggests that some proteins are absent from sympathetically induced secretion or when the glands are becoming partially exhausted. Although more detailed chemical analyses need to be done, the results indicate that not only do sympathetic and vidian nerve stimulations produce different total quantities of protein, but the protein components of the secretions also differ.

Histology

The lateral nasal gland sections contained only serous cells which stained with both PAS and Alcian Blue, as also found by Adams *et al.* (1981). In contrast, Kuijpers *et al.* (1983) reported that in the rat lateral nasal gland three distinct areas are present which contain either neutral, acidic or no glycoproteins.

The complex configuration of electron densities in serous granules in the lateral nasal gland was first reported in the dog by Adams *et al.* (1981) and in the rat by Kuijpers *et al.* (1983). Simpler arrangements occur in bronchial submucosal glands (Bowes & Corrin, 1977; Bowes, Clark & Corrin, 1981) and are related to the distribution of glycoprotein, lysozyme and lactoferrin within the granule. Thus the patterns seen in nasal gland serous granules probably reflect the packaging of proteins and glycoproteins into the granules, and are not fixation artifacts (Kuijpers *et al.* 1983).

The difference in the relative proportions of the various types of granule in stimulated and unstimulated glands presumably reflects the depletion of mature granules and their replacement in the stimulated gland by newly synthesized granules which are rapidly secreted.

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