The influence of nerves on the secretion of immunoglobulin A into submandibular saliva in rats

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1. The influence of sympathetic and parasympathetic nerve stimulations on salivary secretion of immunoglobulin A (IgA) was studied in the submandibular glands of anaesthetized rats by stimulating the nerve supplies with bipolar electrodes.

2. Although the flow of saliva from sympathetically stimulated glands was only 23% of that from parasympathetically stimulated glands the output of IgA was over 2-fold greater. This difference was attributable to influences of the nerves on IgA secretion through the epithelial cell polymeric immunoglobulin receptor-mediated pathway, as Western blotting with specific antibodies to IgA and secretory component revealed that secretory IgA (SIgA) dominated in all saliva samples.

3. Study of saliva secreted in sequential periods of nerve stimulation or following rest pauses suggested that SIgA secretion occurred in the absence of stimulation but this was upregulated 2·6- and 6-fold by parasympathetic and sympathetic nerve stimulations, respectively, compared with the calculated unstimulated rate.

4. The IgA content of extensively stimulated glands was 77% of levels in unstimulated contralateral control glands despite a secretion into saliva equivalent to almost 90% of the glandular IgA content. The IgA may be synthesized and secreted by glandular plasma cells at a rate which exceeds demand and/or such synthesis may be upregulated by nerve impulses.

5. The results indicate that salivary secretion of SIgA is upregulated by nerve impulses and that sympathetic nerves induce a greater effect than parasympathetic nerves.

Secretory immunoglobulins are present in salivary secretions and, along with other glycoproteins such as mucins, lactoferrin and lactoperoxidase, are responsible for helping to maintain the integrity of mucosal surfaces against infectious agents (Mandel, 1989; Underdown & Mestecky, 1994). The predominant secretory immunoglobulin on mucosal surfaces is immunoglobulin A (IgA) and in the mouth it is a component of the salivas secreted by the major and minor salivary glands (Brandtzaeg, 1998).

The submandibular glands are one of three pairs of major salivary glands which secrete saliva into the mouth. Secretion of saliva by these glands is dependent upon signals from parasympathetic and sympathetic autonomic nerves, which release the neurotransmitters acetylcholine and neuropeptides such as vasoactive intestinal polypeptide (VIP) and substance P, or noradrenaline and neuropeptides such as NPY, respectively. These autonomic nerves are the effector arms of reflexes activated mainly by taste and chewing (Gjörstrup, 1980; Matsuo & Yamamoto, 1989). In the absence of stimuli from the efferent nerves, for example under anaesthesia, saliva is not secreted from the rat submandibular gland.

Most salivary proteins secreted by salivary glands are derived from parenchymal cells by exocytosis of protein storage granules across the apical membrane of these polarized cells (Castle *et al.* 1975). In the rat submandibular gland sympathetic nerves cause a profound exocytosis from parenchymal cells whereas parasympathetic impulses have a lesser effect (Garrett *et al.* 1991). Salivary proteins from some submandibular secretory cells have also been demonstrated to enter saliva from a non-storage granule pathway, commonly referred to as constitutive secretion (Pratt *et al.* 1988) which operates both in the absence and presence of stimulation and has been shown to be upregulated by nerve-mediated stimuli (Garrett *et al.* 1996*b*). On the other hand IgA is the product of plasma cells and is secreted initially into the interstitial matrix of salivary glands as polymeric IgA in a complex with J (joining) chain (Brandtzaeg, 1998) and thence enters saliva as secretory IgA (SIgA), a complex of polymeric IgA and the epithelial cell-derived secretory component (SC). SC is the cleaved product of the epithelial cell polymeric immunoglobulin receptor (pIgR) which is crucial to the movement of IgA across secretory epithelia as a consequence of transepithelial

transport, a multi-step process which has been well characterized *in vitro* in pIgR-expressing cell lines (Mostov, 1994). Upon secretion from plasma cells polymeric IgA binds to the pIgR on the basolateral membrane of epithelial cells and then the pIgR–IgA complex is internalized and transcytosed across the epithelial cell to the apical membrane where it is proteolytically cleaved and SIgA is released into saliva (Brandtzaeg *et al.* 1994).

Although impulses from autonomic nerves profoundly influence salivary secretion the effect of nerve impulses on the secretion of IgA into saliva has received little attention previously. The purpose of the present investigation was to study the effects of parasympathetic or sympathetic nerve stimulations on the movement of IgA into submandibular saliva in rats.

METHODS

Overview of experimental protocols

Previous studies have shown that continuous stimulation of sympathetic nerves to rat submandibular glands is usually accompanied by damage to the glands and a diminishing secretion (Anderson *et al.* 1988). A convenient stimulation protocol was developed by applying the stimulation in bursts of high frequency (1 s every 10 s). Even greater fluid and secretory protein outputs can be achieved if the sympathetic stimulation procedure is interupted periodically (Garrett *et al.* 1996*a*), hence the use of such a protocol for sympathetic stimulation in the present work. No such advantages accrue from delivering the parasympathetic stimulation to the submandibular gland in high frequency bursts (authors' unpublished observation). Therefore continuous parasympathetic stimulation was used in the usual manner. In order to avoid differences resulting from the use of a burst stimulation and continuous stimulation for the different nerves the same number of impulses were delivered over the same time periods for both types of stimulation. Such parasympathetic and sympathetic stimulation was used on rats placed in four different experimental groups as below. In the first two groups the effects of either parasympathetic or sympathetic stimulation were compared. In the third group the effects of different periods of rest on the amounts of IgA secreted into saliva were tested. For this purpose only parasympathetic nerve stimulation was used in order to provide sufficient saliva. The effects of an extended period of stimulation were tested in the fourth group of rats.

Nerve preparation and collection of samples

Eighteen male Wistar (King's strain) rats weighing 250–350 g were used in four groups of experiments under chloralose (80 mg kg^{-1}, I.V.) anaesthesia following induction with pentobarbitone (36 mg kg^{-1}, I.P). In the first group (Group 1) of five animals the sympathetic trunk in the neck was cut and the proximal end placed in a bipolar electrode for stimulation at 50 Hz (2 ms pulse width and 5 V) in bursts of 1 s duration every 10 s for 2 min followed by a 2 min rest pause. The submandibular duct was cannulated with a glass cannula with a capacity of approximately 4 μl and saliva secreted was collected into a weighed vial. This protocol was repeated for a further total stimulation time of 40 min and the saliva secreted was collected sequentially into four separate vials (10 min stimulation each). In a second group of five rats (Group 2) the parasympathetic chorda lingual nerve was cut as deeply as possible, reflected onto the submandibular duct and both duct and nerve were placed on a bipolar electrode. Stimulation was performed at 5 Hz (2 ms, 5 V) continuously and saliva collected in five separate vials for a total stimulation time of 42 min as above.

In a third group of four rats (Group 3) the chorda lingual nerve was stimulated as above for a period of 2 min and submandibular saliva collected. Then the gland was allowed to rest for periods of 5, 15, 30 or 60 min, at the end of which stimulation of the gland was repeated for 2 min again and saliva was collected for each stimulation period.

In a fourth group of four rats (Group 4) the chorda lingual nerve and sympathetic chain were prepared. First the sympathetic chain was stimulated as in Group 1 above, then the chorda lingual nerve was stimulated with rest pauses as in Group 3 and finally the chorda lingual nerve was stimulated as in Group 2 rats above.

In all of these experiments saliva samples were collected into weighed vials and the amounts of saliva secreted determined by weight and assuming a specific gravity of 1·0 g ml^{-1} for saliva expressed as volumes. At the end of the above experiments stimulated and the contralateral unstimulated submandibular glands were removed from rats, weighed and then divided into pieces and stored at −70 °C or fixed for morphological analysis (see below). Pieces of tissue for biochemical analysis were thawed and then homogenized using an Ultra Turrax blade disperser set at 20 000 r.p.m.

All of the experiments were conducted under Home Office guidelines and were covered by the necessary licences. At the end of the experiments rats were killed with an overdose of pentobarbitone.

Collection of bile and purification of secretory IgA

At the end of the nerve stimulation experiments described above in four rats the bile duct was cannulated with narrow bore plastic tubing and bile collected into vials placed on ice. In order to purify SIgA 1 ml aliquots of bile were placed on a gel filtration column (Sephadex 200; Pharmacia Biotech Ltd, St Albans, UK) equilibrated with 0·1 M phosphate-buffered saline (PBS). IgA-containing fractions were identified by ELISA as described below except that a purified rat myeloma IgA (Zymed, San Diego, CA, USA) as a standard. Those fractions containing IgA in the molecular mass (as determined by standard proteins) range 350–500 kDa were pooled and concentrated. The IgA content of the concentrate was assayed for protein and 10 μg separated by non-reducing SDS–PAGE. Western blotting with antiserum to secretory component (SC; see below) was used to confirm that the only band present was SIgA.

Immunocytochemistry of IgA

Following removal of the submandibular gland, small pieces of glandular tissue were immediately frozen in hexane cooled on solid carbon dioxide. Ten micrometre frozen sections were cut in a cryosat at −20 °C and incubated with normal rabbit serum followed by fluorescein isothiocyanate (FITC)-labelled rabbit anti-rat IgA (Serotec Ltd, Oxford, UK), diluted 1 in 50 with normal rabbit serum. Negative controls (minus antiserum) and positive controls (stomach mucosa) were incorporated into the study. Sections were mounted on glass slides and then antibody binding was viewed by fluorescence microscopy.

Enzyme-linked immunosorbant assay (ELISA) of IgA

The levels of SIgA present in different salivas were quantified by ELISA. This was performed on 96-well microtitre plates (Griffiths & Neilson, Billingshurst, UK) which were coated overnight at 4 °C

with rabbit anti-rat IgA (Serotec Ltd) diluted 1 in 2000 with 0·1 M carbonate buffer, pH 9·6. Plates were washed 3 times in 0·1 M PBS containing 0·1% Tween 20 (PBS–T) followed by water and then samples were placed on the coated plates and serially diluted in PBS–T. Samples were incubated on the plates for 2 h at 37 °C then the plates washed as above. SIgA purified from rat bile (see above) was quantified by protein assay (Peterson, 1977) and was used as a standard for quantifying the SIgA content of samples. Horseradish peroxidase-labelled rabbit anti-rat IgA antibodies (Serotec Ltd), diluted 1 in 2000 in PBS–T, were incubated on the plate for 1 h at 37 °C. Following incubation, plates were washed then incubated at room temperature in the peroxidase substrate tetramethyl benzidine (Sigma) at a concentration of 3 mg ml^{-1} in DMSO and diluted 1 in 20 in 0·1 M acetate buffer, pH 5·5. Absorbance was measured at 450 nm in an automated microplate reader (BioRad Labs Ltd, Hemel Hempstead, UK).

SDS–PAGE and immuno-detection of IgA

Saliva samples were loaded onto 10% SDS–PAGE separating gels and electrophoresis was performed in the presence of SDS as previously described (Laemmli, 1970) except that samples were not reduced. Resolved proteins were electroblotted onto 0·45 μm nitrocellulose membranes (Anderman & Co., Kingston Upon Thames, UK) for 60 min as previously described (Towbin *et al.* 1979) using a 'wet-blot' apparatus (Transblot, BioRad Labs Ltd) set at 100 V and a limiting current of 1 A. Blotted proteins were detected using FITC as previously described (Carpenter *et al.* 1996). IgA was detected using peroxidase-labelled rabbit anti-rat IgA diluted 1 in 200 with Tris-buffered saline containing 0·1% Tween 20. SC was detected using unlabelled rabbit anti-rat SC (Universal Biologicals Ltd, Stroud, UK) followed by biotinylated goat anti-rabbit antibody (Sigma) and avidin–biotin complex (Vector Laboratories Ltd, Peterborough, UK). Antibody binding bands were detected using enhanced chemiluminescence (ECL, Amersham International) recorded photographically on X-ray film (Hyperfilm, Amersham International) as previously described (Carpenter *et al.* 1996).

Results are expressed as means ± S.E.M. All the statistical comparisons were made using Student's unpaired or paired t tests and P values $< 0·05$ were considered significant.

Table 1. Comparison of mean salivary flow, concentration and output of IgA on electrical stimulation of sympathetic and parasympathetic nerve supplies to the submandibular gland of anaesthetized rats (omitting first samples)

Stimulation	n	Salivary flow rate (μl g^{-1} min^{-1})	Salivary IgA concentration (μg ml^{-1})	Salivary output of IgA (μg g^{-1} min^{-1})
Sympathetic	5	30·6 ± 7·5*	573·0 ± 90·1*	16·4 ± 0·9*
Parasympathetic	5	134·9 ± 24·9	55·8 ± 10·6	7·3 ± 1·1

* Values were statistically significantly different ($P < 0·05$) *versus* parasympathetic stimulation.

RESULTS

IgA content of nerve-evoked salivas

Electrical stimulation of the sympathetic or the parasympathetic nerve supplies to the submandibular gland in anaesthetized rats produced very different volumes of saliva as shown in Table 1. The mean flow of saliva on parasympathetic stimulation was more than 4-fold greater than that on sympathetic stimulation and this difference was consistently observed throughout the study. The IgA concentration of sympathetic saliva was approximately 10-fold greater than that in parasympathetic saliva (see Table 1). However, when expressed as output per unit stimulation time per gram wet weight of gland, the IgA secretion on sympathetic stimulation was approximately 2-fold greater than that on parasympathetic stimulation (Table 1). In order to examine if IgA levels in parasympathetically and sympathetically evoked salivas were consistent, sequential periods of stimulation were performed and the output of IgA in each period was

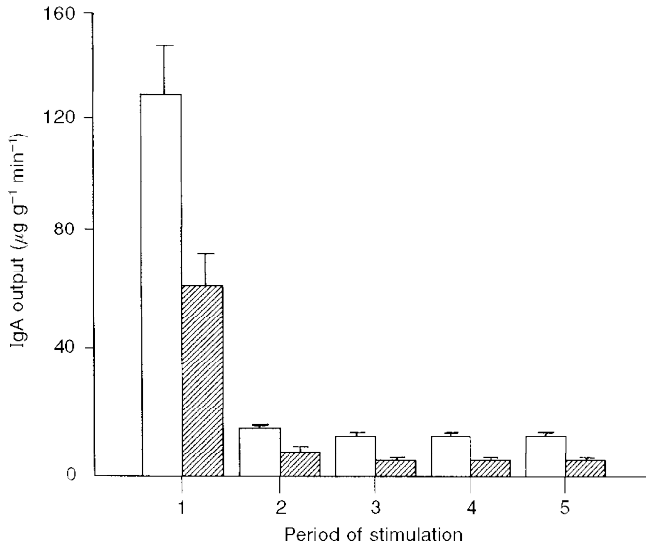

Figure 1. Output of IgA into submandibular salivas during sequential periods of stimulation of either the sympathetic or parasympathetic nerve supplies to the submandibular glands of anaesthetized rats

Sympathetic stimulation (□) was performed in five rats at 50 Hz in bursts of 1 s in 10 s and parasympathetic stimulation (▨) in five rats at 5 Hz continuously. Stimulation in each period was for 10 min except period 1 which was for 2 min. The IgA contents of samples obtained in the first periods of stimulation were greater than those assayed subsequently ($P < 0·05$) for both parasympathetic and sympathetic stimulation. The IgA content of sympathetic saliva was always greater than that of parasympathetic saliva ($P < 0·05$).

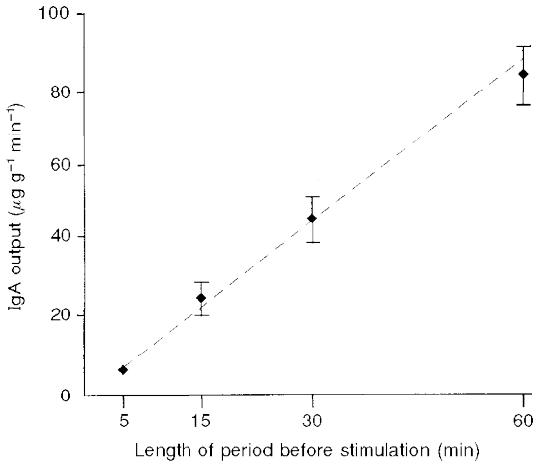

Figure 2. The effects of different periods of rest on the secretion of IgA in submandibular saliva subsequently evoked by parasympathetic nerve stimulation in four anaesthetized rats

The rats had received 2 min of parasympathetic nerve stimulation at 5 Hz prior to each rest period. Secretion of IgA was measured after continuous parasympathetic nerve stimulation at 5 Hz for 2 min after each rest period. There is a progressive, nearly linear increase in the IgA content of saliva with increasing periods of rest.

determined (Fig. 1). IgA secretion in the first period of stimulation following anaesthesia was always much greater than the output in subsequent periods (2–5), regardless of type of stimulation, but the amounts secreted thereafter were always higher in sympathetic saliva.

Accumulation of IgA within submandibular glands

The data in Fig. 1 which show a high concentration of IgA in the saliva collected during the first period of stimulation suggest that IgA accumulates in the gland under anaesthesia, in the absence of stimulation. The accumulated material might then be expelled within the first period of stimulation. As the period of anaesthesia before the first stimulation varied between individual animals depending upon the time taken to prepare the nerves for stimulation, this hypothesis needed to be tested more precisely. For such purposes electrical stimulation of the parasympathetic nerve was performed for 2 min (see Group 3 in Methods) in order to 'clear out accumulated IgA'. The gland was then left for different periods of time and parasympathetic nerve stimulation repeated and saliva collected. The results of this experiment are shown in Fig. 2. As the length of the rest period was extended it is seen that a nearly linear increase in the amount of IgA secreted into saliva occurred, suggesting that a steady accumulation of IgA occurs within glandular lumina with time.

The molecular form of IgA in saliva

The mechanism by which IgA enters saliva should determine its molecular form, that is SIgA, polymeric IgA or monomeric IgA. This was investigated by performing Western blotting using specific antibodies for the rat secretory component or rat IgA and the results are shown in Fig. 3. IgA was present almost exclusively as a species with a molecular mass of $\gg$200 kDa, consistent with it being SIgA which has a predicted molecular mass of 370 kDa. This was confirmed by the binding of an anti-secretory component to the same band and by the fact that purified SIgA from bile had an almost identical mobility (data not shown). Although other anti-IgA-binding bands were

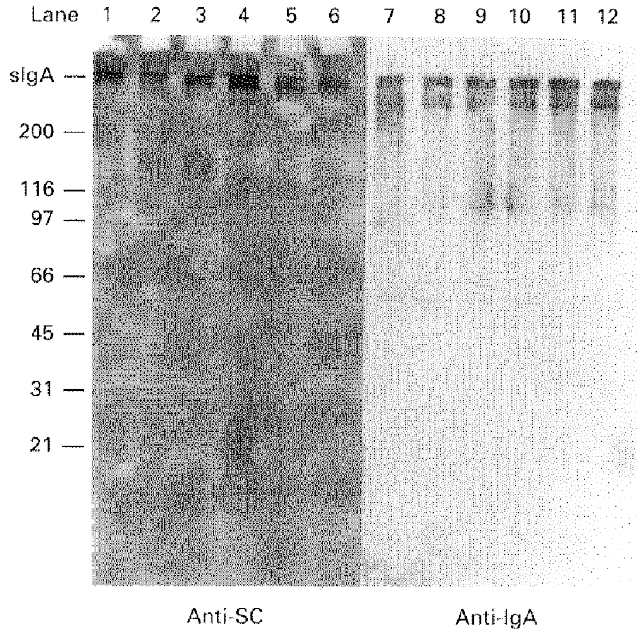

Figure 3. Detection of the secretory component and IgA in samples of sympathetic and parasympathetic salivas following SDS–PAGE under non-reducing conditions

Secretory component, lanes 1–6; IgA, lanes 7–12. Saliva evoked by sympathetic stimulation, lanes 1–3 and 7–9; saliva evoked by parasympathetic stimulation, lanes 4–6 and 10–12. Resolved proteins were electroblotted onto nitrocellulose membrane and probed with antisera. The mobilities of standard proteins are indicated in kDa to the left of the figure and it is clear that the major band detected in all blots has a molecular mass $\gg$200 kDa, consistent with its identity as SIgA.

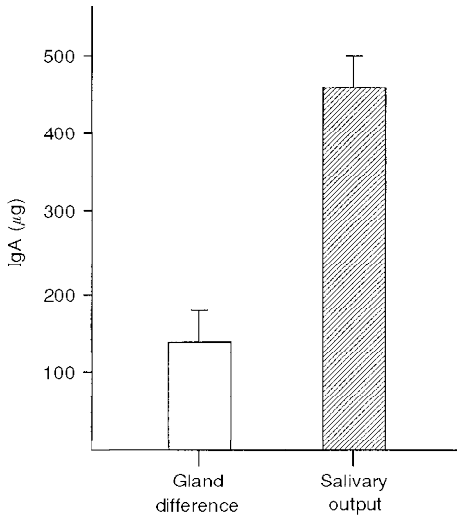

Figure 4. The mean difference in IgA content of unstimulated and contralateral stimulated glands (Gland difference) from four rats (Group 4 in Methods) compared with the mean amount of IgA secreted into saliva

The stimulation protocol was as follows: 42 min of sympathetic stimulation at 50 Hz in bursts of 1 s in 10 s followed by parasympathetic nerve stimulation at 5 Hz for 2 min following rests of 5, 15, 30 and 60 min and finally 42 min of parasympathetic nerve stimulation at 5 Hz. The results demonstrate that the output of IgA into saliva increases as a result of nerve stimulation and is not accounted for by a change in glandular content of IgA. Student's t test indicated a statistically significant difference ($P < 0{\cdot}05$) between decrease in glandular content and salivary output.

present these formed very minor bands in comparison to SIgA and were present in all salivary samples.

Reduction in the glandular content of IgA

To test the effect of either parasympathetic or sympathetic nerve stimulation on glandular IgA levels in submandibular glands a fourth group of rats received stimulation of first the sympathetic and then the parasympathetic nerve supplies over an extended period of time. Firstly, the sympathetic nerve was stimulated at 50 Hz in bursts for 42 min, then the parasympathetic nerve was stimulated at 5 Hz for 2 min following rest pauses of 5, 15, 30 and 60 min and finally the parasympathetic nerve was stimulated at 5 Hz for a period of 42 min. When the IgA contents of stimulated and contralateral unstimulated glands were determined it was found that a mean reduction in IgA of approximately 23% of the total content of the control gland had occurred as a result of stimulation (Fig. 4). The output of IgA into saliva during nerve stimulation of the same glands was 87% of the total content of the control gland. The morphological distribution of IgA within stimulated and unstimulated contralateral control glands was examined by immunocytochemistry. IgA-containing plasma cells were present in all glands and were the most intensely stained structures with small amounts of staining of the interstitial connective tissue around acini and ducts (Fig. 5). There was no visible

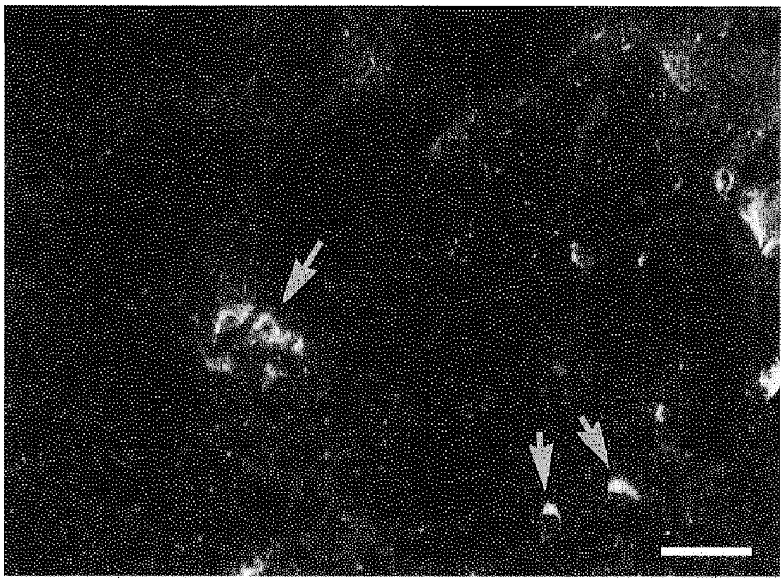

Figure 5. Distribution of IgA in a cryostat section (10 μm) of an unstimulated submandibular gland stained with FITC-labelled antiserum to rat IgA and viewed by fluorescence microscopy

IgA is present in plasma cells (indicated by arrows) which were frequently present in the interstitial matrix next to acini or ducts. Some smaller spots of autofluorescence which appeared orange under the microscope are seen in the background. The pattern and intensity of IgA staining was not noticeably different in sections of parasympathetically stimulated or sympathetically stimulated glands. Scale bar, 20 μm.

staining within ductal or acinar cells and there appeared to be little change in the distribution or intensity of anti-IgA staining in stimulated glands (not shown) compared with control glands.

DISCUSSION

Nerve-mediated stimuli that regulate protein secretion from submandibular salivary parenchymal cells by exocytosis have been found to be dominantly sympathetic in rats (Garrett *et al.* 1991). More recently, other protein secretory pathways, the constitutive, constitutive-like and minor regulated secretory pathways, have been found to operate in salivary secretory epithelial cells (Castle & Castle, 1996) and are under the influence of nerve-mediated stimuli (Pratt *et al.* 1988; Garrett *et al.* 1996*a*, *b*; Castle & Castle, 1996). In the present study we have found that nerves also influence the movement of IgA into saliva collected from the submandibular duct. Sympathetically mediated stimuli caused more than a 2-fold greater output of IgA into saliva than did parasympathetic stimulation. IgA is not a secretory product of epithelial cells but its transcellular transport into saliva is dependent upon the epithelial receptor pIgR (Brandtzaeg *et al.* 1994). Thus it is likely that nerve-mediated stimuli increase IgA secretion by exerting an influence on epithelial cells. Cultured plasma cells have been demonstrated to increase IgA production in response to neuropeptides such as substance P (Scicchitano *et al.* 1988), which is present in parasympathetic postganglionic nerves within rat salivary glands. However, increased synthesis and secretion of IgA by plasma cells is likely to take some time to become apparent as increased salivary levels of IgA and is therefore unlikely to directly account for the immediate increased secretion of IgA elicited by nerve stimulation in the present study.

In the complete absence of nerve stimulation, under anaesthesia, we have found that IgA accumulates steadily within a glandular compartment. A similar accumulation in glandular lumina was found previously in our studies of the constitutive secretion of salivary tissue kallikrein by submandibular glands (Garrett *et al.* 1996*a*, *b*). The latter is considered to indicate that the enzyme continues to be secreted from the apical side of the cells in the absence of fluid secretion. When parasympathetic nerve stimulation was then performed this accumulated material was 'flushed out' of the ductal system by the flow of saliva. IgA appears to accumulate similarly within the luminal system of the submandibular gland. Transcytosis of IgA is likely to continue during anaesthesia in the absence of nerve stimulation. This is suggested by studies on polarized Madin–Darby Canine Kidney (MDCK) cells transfected with the gene for human pIgR in which the binding of polymeric IgA to the receptor has been found to be a stimulus for transcytosis (Cardone *et al.* 1996). It seems that in the absence of nerve stimulation transcytosis is operating at a basal rate which can be calculated from Fig. 2 as being $1{\cdot}7 \pm 0{\cdot}2\ \mu g\ g^{-1}\ min^{-1}$. This basal secretion of IgA in the absence of salivary secretion is responsible for the accumulation of IgA within the ductal system of the submandibular gland and the high initial outputs of IgA measured upon stimulation following anaesthesia (see period 1 in Fig. 1). Comparison of the basal output of IgA (calculated from the gradient in Fig. 2) with those outputs observed during parasympathetic and sympathetic stimulation, $4{\cdot}4 \pm 0{\cdot}8$ and $10{\cdot}3 \pm 1{\cdot}2\ \mu g\ g^{-1}\ min^{-1}$, respectively (see periods 3, 4 and 5 in Fig. 1), suggests that both types of nerve stimulation increase the movement of IgA into saliva, although clearly this effect is greater with sympathetic stimulation.

It could be that sympathetic and parasympathetic stimuli upregulate the transcytosis of IgA. MDCK cell transcytosis is stimulated by phosphorylation of pIgR (Mostov, 1994). The involvement of the inositol trisphosphate (IP_3)–protein kinase C (Cardone *et al.* 1996) and the cAMP–protein kinase A (Hansen & Casanova, 1994) pathways in such stimulation has been demonstrated. Sympathetic and parasympathetic responses within salivary acinar epithelial cells involve these second messenger pathways (Baum, 1987; Quissell *et al.* 1992) and so the phosphorylation of pIgR might also occur during sympathetic and parasympathetic stimulation resulting in an increased rate of transcytosis. That nerves are exerting an influence predominantly through pIgR-mediated transcytosis is supported by the observation that IgA was predominantly present as SIgA in all saliva samples.

Assay of the IgA content of glandular homogenates demonstrated that a small reduction in IgA occurs in extensively stimulated glands compared with unstimulated glands, excised immediately after stimulation ceased. When the total output of IgA into saliva was determined it was found to be much greater than can be accounted for by the reduction in glandular content (Fig. 4). This suggests that IgA is being synthesized and secreted continuously at a rapid rate by plasma cells within the gland and that this rate increases in stimulated compared with unstimulated glands. Axons can be found in close proximity to plasma cells in salivary glands (Garrett, 1999) and thus may influence plasma cell IgA secretion although such morphological evidence is only circumstantial. However, IgA production and secretion by plasma cells may always be in excess of demand and the excess drained from the gland through lymphatic vessels. In times of increased demand less IgA might be drained into the lymphatics and more transported into saliva by the epithelial cell pIgR. Further experiments will be required to determine if nerve-mediated stimuli can directly or indirectly influence the rate at which IgA is produced by plasma cells in salivary glands.

In conclusion, the present *in vivo* results suggest that, in resting unstimulated rat submandibular glands there is a continuous synthesis of IgA by plasma cells accompanied by transcellular movement across the cells to lumina at moderate basic rates. The study also demonstrates that both types of autonomic nerve stimulation increase the output of SIgA into submandibular saliva and this is thought to relate to

metabolic changes in the glandular epithelial cells. Sympathetic neurotransmitters appear to have proportionately greater effects on this process under the present conditions than those from parasympathetic nerves. Morphological studies of the autonomic innervation (Garrett & Kidd, 1993) and plasma cells (Brandtzaeg, 1998) of human submandibular and parotid salivary glands along with functional studies of human salivary cells *in vitro* using autonomic agonists and antagonists (Segawa *et al.* 1998) suggest that autonomic control of human salivary secretion might be exerted in a similar way to that observed in the rat. It may be, therefore, that human submandibular and parotid salivary levels of IgA can be altered through autonomic activation. Since submandibular and parotid glands contribute over 80 % of the saliva present in the mouth (Dawes, 1987) it is likely that such autonomic influences would significantly elevate levels of SIgA in the mouth.

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