

Differential Effect of Autonomic Stimulation on Salivary Secretion of IgG, IgA and Amylase (40139)

SHERIF EL-MOFTY¹ AND CHARLOTTE A. SCHNEYER

Department of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, Alabama 35294

The presence of proteins and glycoproteins in salivary secretion is well documented (1). These organic components can be distinguished into two general classes. Some are serum proteins, others are intrinsic secretory materials manufactured in the salivary gland. The mechanism of secretion of these different components is not clearly understood and only few studies have dealt with the influence of the nature of the secretory stimulus on secretion of proteins in saliva. Secretion of some of the intrinsic proteins, e.g., amylase has been shown to be dependent on autonomic stimulation (2). However, the role of autonomic stimulation in regulating secretion of serum proteins has not been delineated. A convenient method for collecting saliva and simultaneously evaluating the effect of sympathetic and parasympathetic stimulation involves the use of pilocarpine stimulation of an acutely sympathectomized parotid gland (Sx-Pc) and pilocarpine stimulation of the intact contralateral mate (Pc). This procedure permits analysis of parasympathetic and sympathetic influences on one hand (intact pilocarpine stimulation), and parasympathetic influence on the other hand (acute Sx-pilocarpine stimulation) (2).

In the present study this system was used to collect parotid saliva from the paired glands of the same animal. The purpose was to examine the secretion of several parotid proteins in response to different stimuli. This was attempted by measuring concentrations of amylase, IgG, IgA and total protein in saliva. Moreover, it was hoped that a comparison of secretory responses might reveal differences in modes of transport and thereby provide information on glandular immunoglobulin transmission. Determination of the influence of flow rate was considered important in standardizing quantitative values.

Materials and methods. Adult male rats (4-6 months old) of randomly bred Long-Evans strain were used in these experiments. Animals were fasted but given water during the 24 hr prior to an experiment. For an experiment, rats were anesthetized with pentobarbital (50 mg/kg body wt), the superior cervical ganglion was unilaterally removed and ducts of both parotid glands were exposed and cut (3). Flow of saliva was evoked by injection of supramaximal dose of pilocarpine (3 mg/300 g animal, ip). Saliva was collected by micropipette applied to the orifice of the duct. Rate of flow of saliva was obtained by measuring the volume of saliva secreted per minute and relating this to the wet weight of the gland producing the saliva. Saliva was collected continuously, with samples taken at timed intervals.

Amylase of saliva was determined by the method of Myers, Free and Rosinski (4), using samples properly diluted with phosphate-buffered saline, and expressed as mg of reducing substance formed during the 15-min digestion period per mg parotid saliva. Total protein was determined using the Folin-Ciocalteu reagent (5) and protein concentration was expressed as grams of crystalline bovine albumin. The IgG and IgA contents of saliva were quantitated by radial immunodiffusion (6) using commercial rat IgG and rabbit anti-rat IgG (Miles Labs, Kankakee, IL). The immunodiffusion was carried out immediately after collection of the samples. Rat IgA was separated by rivanol-ethanol fractionation of colostrum (7) and rabbit antibody to rat IgA was prepared by repeated multiportal immunization of rabbits with colostral IgA in complete Freund's adjuvant. The monospecificity of the antisera to IgG and IgA heavy chains was tested by immunodiffusion and immunoelectrophoresis.

Results. When one superior cervical ganglion was extirpated and pilocarpine was administered, samples of parotid saliva were

¹ Present address: Dr. Sherif El-Mofty, Faculty of Dentistry, Cairo University, Cairo, Egypt.

obtained from innervated and denervated glands in the same animal. As shown by data in Fig. 1, the flow rate of rat parotid saliva

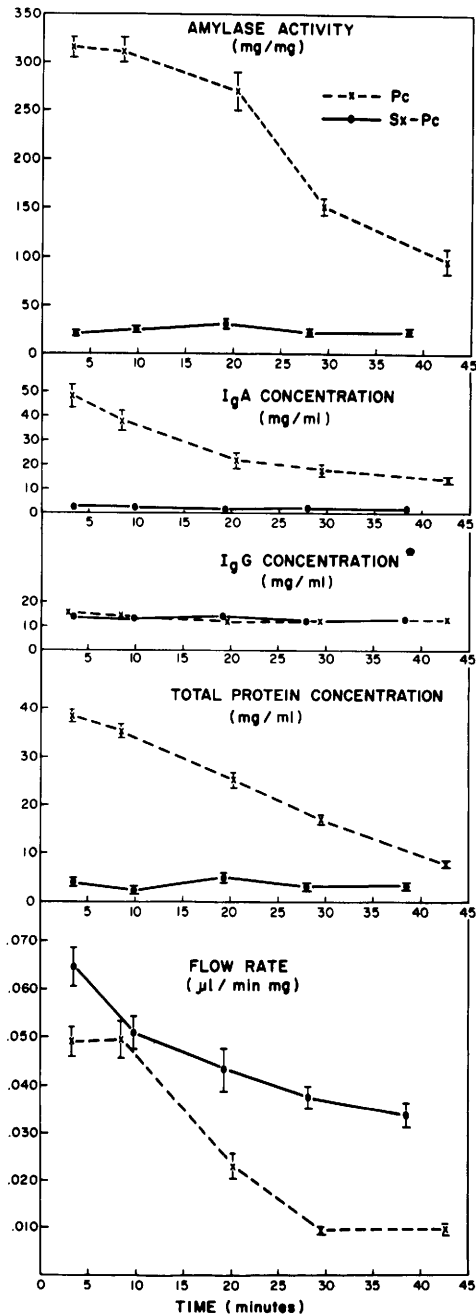


FIG. 1. Flow rate, total protein, IgA and IgG concentrations in rat parotid saliva of sympathectomized and neurally intact contralateral glands. Each point is the mean \pm SE obtained from at least eight animals.

was high immediately following stimulation and gradually decreased with time. Pilocarpine was more effective in eliciting secretion of saliva in the sympathectomized gland. During 45 min following stimulation the flow rate of saliva from the neurally intact parotid gland (Pc) was strikingly lower than that of the sympathectomized mate (Sx-Pc). The difference between flow rates of the neurally intact and sympathectomized parotid glands increased with time, reaching a maximal difference by 30 min. This level of difference was thereafter maintained. The total protein values of saliva collected from the neurally intact (Pc) gland were very much greater (nearly ninefold) than those of the contralateral sympathectomized gland (Sx-Pc) ($P < 0.001$). The mean total protein concentration of the neurally intact parotid gland showed a gradual decrease with time from 38.4 mg/ml immediately following initiation of stimulation to 8.0 mg/ml after 45 min. The total protein concentration of the sympathectomized gland was not appreciably altered with time and remained at very low levels of about 4 mg/ml. After 45 min, the protein levels of saliva from the innervated and denervated glands were similarly low. The total protein level per volume of secreted saliva remained more or less constant irrespective of the flow rate of saliva collected from the sympathectomized gland. The level of amylase activity in parotid saliva collected initially (first 5–8 min) averaged 314 mg reducing sugar per mg of saliva (mg/mg) when pilocarpine was used to evoke secretion from neurally intact gland. The level of amylase gradually decreased with time reaching an average value of 97 mg/mg after 45 minutes. The most pronounced drop of amylase activity occurred between 20 and 30 minutes following pilocarpine injection (270 mg/mg to 152 mg/mg). The amylase levels in secretion obtained from the sympathectomized parotid gland were very much lower than the levels obtained from the normally innervated mate, and initially, were only one-twentieth that of the intact gland. Furthermore, there was little change in magnitude with time or with level of flow rate, and levels of amylase of saliva from the sympathectomized gland were consistently low throughout the 45 min.

The pattern of IgA in the secretions of these two fluids (Sx-Pc) and (Pc) differed

from each other. The level of IgA concentration in parotid saliva from the neurally intact side averaged 48 mg/100 ml immediately following stimulation and gradually decreased to 14 mg/100 ml after 45 min. After extirpation of the superior cervical ganglion, levels of IgA of pilocarpine evoked saliva were about 5 mg/100 ml and did not change appreciably with time or with level of flow rate. On the other hand, measurement of IgG concentration in saliva from the denervated side showed no statistically significant difference from that of the innervated gland in the same animal. Furthermore, the levels of IgG did not change appreciably with time or rate of flow in both groups.

Discussion. The present data clarify the role of the autonomic innervation in regulating salivary protein secretion. Previous studies have shown that stimulation of adrenergic receptors caused a marked secretion of protein from the gland, either into saliva (8, 9), blood (10) or *in vitro*, into medium (11). In the present study, evaluation of the effects of sympathetic and parasympathetic stimulation was undertaken. The effects caused by adrenergic glandular receptors could be separated from those produced by cholinergic receptors on the gland simply by comparing pilocarpine-induced secretion in the neurally intact gland with that induced in the acutely sympathectomized gland (2).

In confirmation of previous work (12), levels of amylase activity and concentrations of total protein in saliva of rat parotid gland evoked by administration of supramaximal doses of pilocarpine showed striking differences from those of saliva of the sympathectomized contralateral gland. A relationship between levels of amylase in the gland and those in saliva has been shown to exist, and the extent of the glandular depletion and amount appearing in saliva is also related to the kind of autonomic stimulation employed (12). In this regard adrenergic stimulation is the most effective in causing secretion of amylase into saliva and depletion of glandular contents of amylase. It has been shown specifically to promote release of amylase from the acinar cells (13). As a result of the effectiveness of sympathetic stimulation, the amylase level was very high immediately following stimulation and gradually decreased due to depletion of glandular contents of

amylase. On the other hand, cholinergic stimulation resulted in secretion of saliva with low amylase content, and the amylase level did not change appreciably with time, reflecting the minor role played by this type of stimulation in eliciting amylase secretion into saliva and also in causing glandular depletion (12). The differences in total protein and amylase levels could not be accounted for by changes in flow rate. The secretion of IgA paralleled in pattern that exhibited with amylase and total protein. From this parallelism, it may be inferred that the acinar cells of the parotid gland play a role in the secretion of IgA. However, the extent of the change of IgA concentration with different stimuli was not as extensive as that of amylase, suggesting that the mechanism of secretion of IgA is not identical in all respects to that of amylase. This supports the view that all subunits of secretory IgA are not derived from acinar cells. It is generally agreed that the immunoglobulin moiety of IgA is secreted by immunocytes (14) and becomes conjugated with secretory piece upon passage through the glandular epithelium (15). It is not probable that the subepithelial immunocytes are controlled by the autonomic innervation, but the noted dependency of IgA secretion on autonomic innervation might be through the control of secretion of secretory piece.

In contrast to these findings, IgG levels in rat parotid saliva were independent of flow rate, and kind and time of stimulation. With pilocarpine and low isoproterenol stimulation (16), levels are similarly low. Furthermore, when paired glands, one of which was sympathectomized and the other left neurally intact were stimulated by pilocarpine, the IgG levels of the saliva evoked from both were virtually identical. In previous studies it has been noted that albumin was secreted in saliva in similar fashion and that the ratio of albumin and IgG in saliva was about the same as that in serum (17). These findings suggest that these molecules are passively transported to saliva from the serum.

The evidence, therefore, shows there is separation of secretion of amylase, IgA and IgG, with amylase and IgA being secreted from the acinar epithelium and the IgG being passively transported from blood to saliva without any acinar contribution.

Summary. Stimulation of the rat parotid

gland was effected by pilocarpine administration immediately following removal of one superior cervical ganglion and saliva was collected from the denervated gland and contralateral neurally intact mate. In this fashion it was possible to evaluate the effects of sympathetic and parasympathetic stimulation on the secretory responses of several proteins in parotid saliva. The flow rate of saliva obtained with cholinergic stimulation was higher than that with adrenergic stimulation. On the other hand, stimulation of adrenergic receptors caused secretion of total protein and amylase from the gland, in appreciably higher concentration than that elicited in response to cholinergic stimulation. Amylase level in secretion obtained from the neurally intact parotid gland immediately following pilocarpine injection was about 20 times that of the sympathectomized gland and gradually decreased with time reaching a level of about 4 times that of the denervated mate after 45 min. The levels of IgA in saliva evoked by sympathetic and parasympathetic stimulation paralleled the pattern noted with amylase but the difference with different autonomic stimuli was not as extensive as that with amylase. From this similarity of pattern and difference in extent between the secretion of IgA and amylase, it was inferred that IgA is not transferred by the gland like intrinsic proteins. IgG levels in saliva were independent of flow rate and kind and time of stimulation.

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