J. Physiol. (1979), **296**, *pp.* 443–451
With 5 text-figures
Printed in Great Britain

AMYLASE SECRETION IN THE RABBIT PAROTID GLAND WHEN STIMULATING THE SYMPATHETIC NERVES DURING PARASYMPATHETIC ACTIVITY

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(*Received 15 December 1978*)

SUMMARY

1. In anaesthetized rabbits amylase secretion from the parotid gland was investigated. Secretion was evoked by sympathetic nerve stimulation, either alone or superimposed on a parasympathetic background secretion, imitating the resting secretion present in the waking animal.

2. Sympathetic nerve stimulation at frequencies below 1 Hz was alone subthreshold for fluid secretion, but could greatly increase the amounts of amylase present in fluid secretion produced by parasympathetic nerve stimulation. The amylase output due to sympathetic nerve stimulation alone at 10 Hz did not exceed that seen in response to a stimulation at 1 Hz superimposed on parasympathetic activity.

3. The amylase output in response to superimposed sympathetic stimulation was not influenced by the rate of fluid secretion, which was altered by stimulating the parasympathetic nerves at different frequencies.

4. Sympathetically-evoked amylase secretion was abolished after β_1-block. The amylase secretion remaining on parasympathetic activation was sparse.

5. It is concluded that secretion of amylase in response to sympathetic nerve stimulation requires the presence of a parasympathetic fluid secretion to be washed along the glandular ducts. Parasympathetic activity may also augment the sympathetic effect on amylase secretion.

INTRODUCTION

Experiments on salivary secretion carried out mainly in dogs and rabbits led Heidenhain (1878) to the conclusion that salivary glands receive two kinds of nerves apart from vascular nerves, namely secretory nerves and trophic nerves. The former were supposed to control the secretion of water and electrolytes, the latter the secretion of organic substances. The major protein to be exported from rabbit and rat parotid glands, and thus extensively studied, is amylase. Investigations on the nervous control and the cellular mechanisms of the secretion of amylase have disclosed that the 'trophic' effect in Heidenhain's sense is exerted via sympathetic fibres acting on β-adrenoceptors; some amylase is also seen in response to activation of α-adrenoceptors or stimulation of cholinergic nerves (see Schramm & Selinger, 1975; Garrett & Thulin, 1975). As in other glands the secretion of water and electrolytes in the rabbit parotid gland is mainly controlled by cholinergic nerves, and the

0022-3751/79/5560-0906 $01.50

volumes secreted by sympathetic activation are small (Schneyer & Emmelin, 1974; Gjörstrup, 1977).

In investigations on dogs and cats (Emmelin & Gjörstrup, 1975, 1976), it was found that the sparse fluid secretion obtained on electrical activation of the sympathetic nerves could be greatly augmented and the threshold frequency lowered by the presence of a slow parasympathetic secretion. The same phenomenon, although less pronounced than in the cat and dog, has recently been demonstrated in the rabbit parotid gland (Gjörstrup, 1977). From these observations it was regarded of interest to investigate whether a parasympathetic background secretion is of importance for the amylase secretion elicited by sympathetic nerve stimulation. A preliminary account of some of these results has been published previously (Gjörstrup, 1978).

METHODS

Seventeen rabbits, weighing 2·3–3·5 kg and kept on a standard pelleted diet, were used. 23–25 hr prior to the experiment the animals were deprived of food and placed in cages designed to prevent coprophagia; water was allowed *ad libitum*. The animals were anaesthetized with urethane, 1·5 g as an initial dose, via a lateral ear vein. During the following half hour another 0·5–1·0 g of urethane was given. Elimination of the corneal reflex was the criterion used for the adequacy of anaesthetia and was frequently checked during the experiment and occasionally additional doses of urethan were given. Apart from the first dose of urethane all injections of drugs were made through a cannulated femoral vein. A tracheal cannula was inserted. The right parotid duct was exposed and cannulated, as close to its orifice in the mouth as possible, with a glass cannula giving drops of saliva of a size of 15 μl. The drops were recorded on a smoked drum by an ordinate writer, manually operated. The auriculo-temporal nerve carrying the main parasympathetic nerve supply to the parotid gland (Alm & Ekström, 1976), was dissected as described for rabbits and for cats and dogs by Nawrocki (1868) and Burgen (1964). The sympathetic nerve was dissected in the neck. Both nerves were placed on bipolar platinum electrodes and stimulated at supramaximal voltage and a shock duration of 2 msec, using Grass stimulators, S4 and S44, supplied with stimulus isolation units.

Sympathetic stimulation superimposed on a slow para-sympathetically induced secretion may change the rate of salivary flow in two ways, thereby possibly complicating the interpretations when comparing amylase in samples collected before and during sympathetic stimulation. Vasoconstriction may reduce the flow rate and judging from experiments on the submaxillary gland of the rabbit this complication arises when the sympathetic is excited at frequencies above 1 Hz (Gjörstrup, 1977). Secondly, when the parasympathetically evoked secretion is slow (15–40 μl./min), superimposed stimulation of the sympathetic secretory fibres causes 'augmented' salivary secretion (Gjörstrup, 1977). In preliminary experiments this augmentation disappeared when the flow rate was increased to 50–100 μl./min, which was attained by stimulating the auriculo-temporal nerve at 1–2 Hz. This higher flow rate was then used in a series of seven rabbits, and while the auriculo-temporal nerve was excited, periods of sympathetic stimulation at the following frequencies were superimposed: 0·05, 0·1, 0·3, 0·5 and 1·0 Hz. The schedule for these experiments was as follows: In the continuous parasympathetic stimulation the sympathetic was stimulated at each frequency for a 7 min period and the saliva formed during the last 5 min collected for measurement of amylase; saliva secreted during the first 2 min was discarded because of dead-space and time to reach steady state. Each period with superimposed sympathetic stimulation was preceded by an identical parasympathetic control period. In addition two extra 5 min samples of parasympathetic saliva were collected at the beginning of each experiment. After β-adrenoceptor block the procedure was repeated. Sympathetic nerve stimulation at frequencies higher than 1 Hz was carried out separately in a second series of five other rabbits. In these experiments the purpose was to compare the amylase in samples obtained with sympathetic nerve stimulation alone with those seen during superimposed stimulation. In these experiments the parasympathetic nerves were also stimulated at frequencies between 1 and 2 Hz to produce a flow between 50–100 μl./min.

In a third series, five experiments were carried out to see whether the output of amylase caused by sympathetic impulses depended on the rate of fluid secretion. This rate was varied to five different levels by exciting the auriculo-temporal nerve at frequencies between 0·7 and 7·5 Hz. The amylase secretion caused by sympathetic stimulation at 0·3 Hz was studied at these five flow rates. The basic scheme for collection of samples of saliva was the same as that used in the first series and is given above.

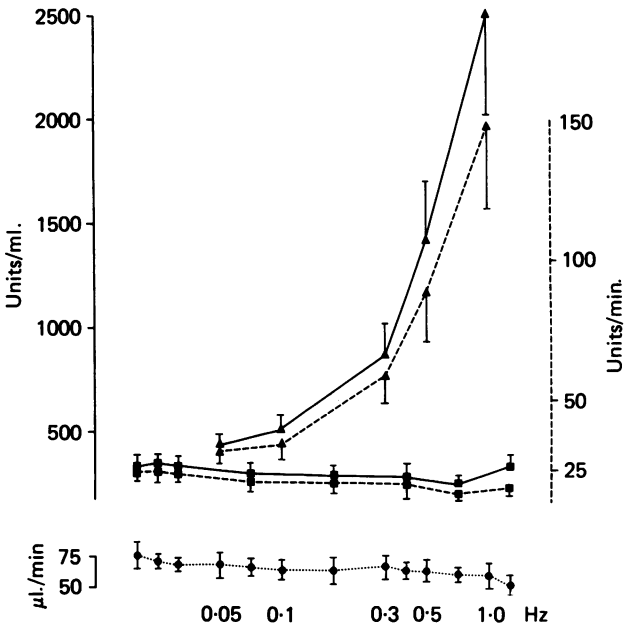

Fig. 1. Amylase secretion in response to sympathetic nerve stimulation superimposed on a continuous flow of saliva evoked by stimulating the parasympathetic nerves at 1–2 Hz. Throughout the experiment samples of saliva for amylase determination were collected as described in Methods. When the flow rate had been adjusted to the desired level (see Methods) sympathetic nerve stimulations at the frequencies given were superimposed a regular intervals and the saliva formed during the stimulation periods collected. Before each period with sympathetic nerve stimulation there was a similar period when only the parasympathetic nerves were stimulated. In addition each experiment was begun by collecting two control samples of parasympathetic saliva. The average rate of fluid secretion for the different periods when saliva was collected is shown (●....●). Amylase secretion, expressed as sample concentration in units/ml. (———) and as output in units/min (– – – –), is reproduced separately for the parasympathetic control samples (■) and for the samples obtained by superimposed sympathetic nerve stimulation (▲). All values are mean ± S.E. of mean from seven experiments.

Amylase measurement

The samples were stored in deep freeze until assayed according to the method described by Dahlqvist (1962). In this method 1 unit of amylase is equivalent to the amount that liberates reducing groups corresponding to 1 µmole maltose formed each minute at 25 °C. Samples of saliva of 50 or 100 µl. were used and diluted 200–1000 times before processing.

Amylase obtained in the saliva is expressed as sample concentration of amylase (units/ml.) and as output of amylase per min (units/min).

Drugs

Practolol (I.C.I.), 4 mg/kg, and atenolol (I.C.I.), 2 mg/kg, were given I.V.

Statistics

Student's t test for paired and unpaired observations and variance analysis of the mean were used. P values less than 0·05 were considered significant.

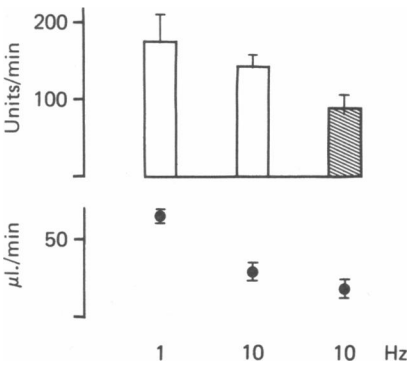

Fig. 2. Sympathetic stimulation at 1 and 10 Hz in the presence of a parasympathetic secretion (open columns) and alone at 10 Hz (hatched); in the two former cases the parasympathetic contribution of amylase has been corrected for to obtain the pure sympathetic effects. Amylase secretion in units/min. Below, the rate of fluid secretion in μl./min (mean $\pm$ S.E. of mean) (n = 5).

RESULTS

Continuous stimulation of the parasympathetic nerves at 1–2 Hz for 1·5 hr evoked a fluid secretion that during the first 5 min had an average rate of 75 ± 13 μl./min, (mean $\pm$ S.E. of mean) (n = 7) which gradually slowed down to 51 ± 9 μl./min at the end of the stimulation period (Fig. 1). The corresponding mean amylase concentrations however remained fairly stable 333 ± 49 and 336 ± 69 units/ml., respectively. Thus the total output of amylase in the parasympathetic control samples came to parallel the secretory rate and their means to range between 24 ± 3 in the first and 15 ± 3 units/min in the last 5-min sample.

When sympathetic nerve stimulation, 0·05–1·0 Hz, was superimposed on the parasympathetically evoked flow of saliva, the secretion of amylase increased in a frequency-dependent manner, while the flow rate remained unchanged. The sample concentration of amylase ranged between 437 ± 55 units/ml. for 0·05 Hz and 2513 ± 480 units/ml. for 1·0 Hz (mean $\pm$ S.E. of mean) (n = 7), and the output of amylase per min between 31 ± 5 units and 147 ± 30 units (Fig. 1). The increases in amylase secretion were highly significant, when compared with the preceding parasympathetic control samples ($P < 0{\cdot}001$). Single shocks of superimposed sympathetic nerve stimulations were not found to increase the secretion of amylase (three experiments).

Sympathetic stimulation alone at 1 Hz was subthreshold in all animals when flow of saliva was used as means to detect secretion. To produce a fluid secretion on

sympathetic stimulation alone, frequencies at or above 3 Hz were used (Gjörstrup, 1977), but only at 10 Hz were the volumes regularly large enough for amylase determinations. At this stimulation frequency a fairly high flow rate of saliva (50–70 ul./min) was obtained during the first 1–2 min, to slow down to be between 10 and 20 ul./min when samples of saliva were collected. Also when sympathetic

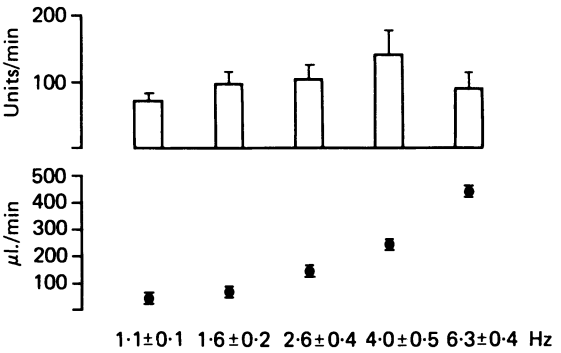

Fig. 3. Sympathetic stimulation at 0·3 Hz, superimposed on different secretory rates, evoked by stimulating the parasympathetic nerves at different frequencies. Columns indicate amylase secretion in units/min and below is shown the rate of fluid secretion in μl./min (mean ± S.E. of mean) (n = 5).

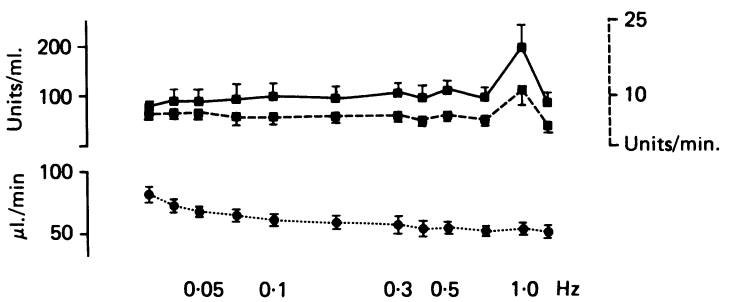

Fig. 4. Parasympathetic stimulation with and without superimposed sympathetic stimulation, after β_1-adrenoceptor block with atenolol. The experimental procedure is given in the text to Fig. 1 and in the Methods. The amylase output is shown as sample concentrations in units/ml. (■——■) and as amylase secretion in units/min (■ - - - - ■). Below are given the secretory rates in μl./min (●. . . . ●) (mean ± S.E. of mean) (n = 4).

stimulation at 10 Hz was superimposed on a parasympathetic secretion the flow rate after a short interval started to decline markedly; drops for amylase determination could be collected at a flow rate around 30 ul./min, compared to the initial flow rate of about 70 ul./min. In both cases the reduced salivary flow rate is attributed to vasoconstriction following sympathetic stimulation (Gjörstrup, 1977). Sympathetic stimulation alone at 10 Hz evoked a lower output of amylase per min than when either 1 Hz (P < 0·02) or 10 Hz (P < 0·02) were superimposed on a parasympathetic secretion. Further, no difference was revealed between the outputs of amylase that were secreted in response to superimposed sympathetic stimulation at 1 and 10 Hz in the same experiment (Fig. 2).

In each of five animals the level of parasympathetic activity was varied to produce an up to tenfold difference in the rate of fluid secretion, 45–450 ul./min. At five different flow rates within this range sympathetic stimulation at a set frequency of 0·3 Hz was superimposed. This procedure did not change the amounts of amylase that were secreted in response to the sympathetic stimulation. At all the flow rates studied the amylase output kept around 100 units/min (Fig. 3).

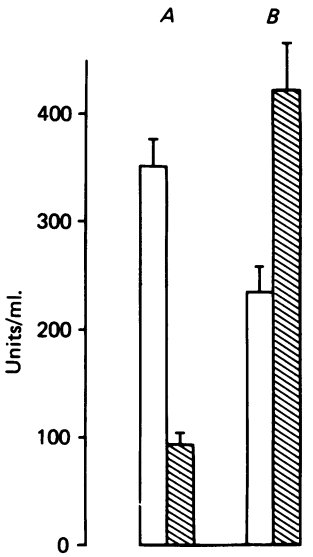

Fig. 5. Amylase concentration in parasympathetic control samples. Under *A* are shown values in all controls before (n = 32) and after (n = 28) atenolol, 2 mg/kg. Under *B* the same is shown for practolol, 4 mg/kg (n = 16 and n = 14, respectively). Open columns indicate before and hatched columns after β-block.

In six of the seven rabbits in the first series, amylase secretion in response to sympathetic stimulation was studied after β-block, atenolol (n = 4) or practolol (n = 2). The procedures for sympathetic and parasympathetic stimulation that were used before β-block were repeated (Fig. 2). Except for a minute response at 1 Hz the effects of all frequencies of sympathetic stimulation were completely abolished (Fig. 4). However, the amylase content in the parasympathetic control samples was affected differently by the two β-blocking agents. It was lowered after atenolol, while it was increased after practolol (Fig. 5).

DISCUSSION

The present experiments show that amylase secreted in response to electrical stimulation of the sympathetic nerves at low to moderate stimulation frequencies requires a background of parasympathetic fluid secretion in order to be transported out into the main duct and thus to reach the mouth. This observation further reveals that the sympathetic threshold frequency for amylase is lower than that for fluid secretion. While the threshold frequency for amylase secretion was 0·05 Hz, that for fluid secretion has been shown to be around 2 Hz at sympathetic stimulation alone

and 0·15 Hz in a parasympathetic background secretion (Gjörstrup, 1977). It is known that in the waking animal a slow reflexly elicited parasympathetic secretion is present (Schneyer & Emmelin, 1974), so there is every reason to believe that if sympathetic excitation normally takes place it should do so against a background of parasympathetic secretion. Thus the present approach for studying the effects of sympathetic nerve stimulation seems to be more physiological than stimulating these nerves separately. That the sympathetic secretory nerves can be reflexly activated during feeding has been demonstrated in the rat (Ohlin, 1968; Harrop & Garrett, 1974; Speirs & Hodgson, 1976). Investigations on the reflex salivary secretion in the dog has shown that sympathetic nerves may take part in the formation of saliva secreted from the submaxillary but not from the parotid gland (see Babkin, 1950).

The amount of amylase secreted in response to a particular sympathetic stimulation (0·3 Hz) was not changed when fluid secretion was varied over a wide range by stimulating the parasympathetic nerves at different frequencies. Presumably a fairly low rate of fluid secretion sufficed for an effective transport of amylase along the duct system. Very low parasympathetic flow rates were not tried because augmented sympathetic fluid secretion is then at hand to increase the flow of saliva. That augmented sympathetic fluid secretion is seen only at a slow parasympathetic secretion in the rabbit parotid gland (see Methods) may indicate that its importance in this gland is to maintain an adequate transport of amylase even if the parasympathetic background activity is low.

Activation of the parasympathetic nerves may not only produce the fluid secretion necessary for the transport of amylase secreted in response to sympathetic stimulation, but may also augment sympathetic amylase secretion. In favour of this hypothesis are the facts that during parasympathetic background activity the sympathetically evoked amylase secretion has a very low threshold frequency and that sympathetic stimulation at 1 Hz causes a secretion of amylase almost of the same magnitude as the maximum obtained when isoprenaline is injected while the parasympathetic nerves are stimulated (B. Asking & P. Gjörstrup, unpublished). Further, the secretion of amylase at 1 and 10 Hz during parasympathetic activity was of the same magnitude, while sympathetic stimulation alone at 10 Hz produced less amylase. However, experiments performed at high sympathetic stimulation frequencies must be interpreted with caution because vasoconstriction may change metabolism and hence alter normal glandular function. The possibility of eliminating the vasoconstriction by blocking the α-adrenoceptors was not used for the following reasons. In rabbits α-block is difficult to establish on the vascular bed, and when fully developed the animals generally tend to have a low blood pressure (Harvey & Nickerson, 1953; P. Gjörstrup, unpublished). Another major drawback is that α-block abolishes most of the fluid secretion seen in response to sympathetic nerve stimulation (Nordenfelt & Ohlin, 1957; Gjörstrup, 1977). The fact that increasing the parasympathetic activity did not increase the amylase secretion to sympathetic stimulation may seem incompatible with the view that there is an augmented amylase secretion. But as in the case for augmented fluid secretion it may be argued that it exists at a very low level of parasympathetic activity and that it already is at a maximum for amylase secretion at the lowest flow rates presently used.

The sympathetically evoked amylase secretion is essentially dependent on activation of β-adrenoceptors since it disappeared after β-block, in accordance with findings in the rat parotid gland (see Schramm & Selinger, 1975, 1976); the selectivity of the block used in the present experiments shows the adrenoceptors to be of the β_1-subgroup (Barrett, Carter, Fitzgerald, Hull & Le Count, 1973; Harry, Knapp & Linden, 1974). The β-block by atenolol showed that parasympathetically evoked saliva by itself has a very low content of amylase. The lowered amylase concentration in parasympathetic saliva after atenolol most likely indicates that during the experiments small amounts of catecholamines are liberated from the adrenals, tending to obscure slightly the phenomenon studied. Due to the proposed interference of circulating catecholamines, β-block ought to be present when amylase secretion in response to parasympathetic nerve stimulation or injection of parasympathomimetic drugs is studied in the rabbit, and very likely also in other species. The observed increase after practolol probably reflects the intrinsic sympathomimetic properties of that agent, and earlier studies on fluid secretion have reported similar findings (Ekström, 1969; Gjörstrup, 1977).

In conclusion it can be said that sympathetic stimulation at frequencies subthreshold for fluid secretion can, provided that a parasympathetic activity is present, evoke almost maximal amylase secretion. The role of the parasympathetic nerves in this context may primarily be to evoke fluid secretion for transport of amylase, but the parasympathetic activity may also in some way serve to augment the sympathetically produced amylase secretion.

This work was supported by grants from the Medical Faculty of Lund, Sweden. Atenolol was kindly supplied by I.C.I.-Pharma, Gothenburg, Sweden.

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