Amylase released from the parotid gland by pilocarpine elevates the enzyme activity in the submandibular and sublingual glands of rats

T. Ikeno, S. Hashimoto and H. Kuzuya

Department of Biochemistry, Tohoku Dental University, Tomita-machi, Koriyama 963 (Japan), 29 December 1980

Summary. Stimulation by pilocarpine led to a marked increase in amylase activity in the submandibular and sublingual glands and serum, and resulted in a considerable depletion of the enzyme in the parotid gland and pancreas of rats. Parotidectomy reduced these pilocarpine effects. It is concluded that amylase released by pilocarpine from the parotid gland but not from the pancreas elevated the enzyme activity in the submandibular and sublingual glands.

Secretion of amylase from the parotid gland and pancreas of rats can be stimulated by both cholinergic and β-adrenergic agents[1,2]. A rapid and considerable increase in amylase activity in the submandibular gland results from short-term stimulation by pilocarpine[3]. The rate of incorporation of ^{3}H-labelled leucine into the parotid gland cells increases after stimulation of amylase secretion by pilocarpine or feeding. In the submandibular gland, however, no increase of incorporation of the labelled leucine is observed after this treatment[4]. Chignell also reported that incorporation of ^{14}C-labelled amino acids into amylase protein in the submandibular gland decreased after the administration of pilocarpine[5]. He also showed that both actinomycin D and puromycin failed to block the pilocarpine-stimulated increase in amylase activity in the submandibular gland. He postulated that amylase activity in the submandibular gland would rise if the rate of degradation of amylase was decreased by the administration of pilocarpine while the rate of synthesis remained the same. We report here a mechanism for increase of the amylase activity in the submandibular and sublingual glands of rats by the administration of pilocarpine.

Materials and methods. Male Wistar rats, weighing 150–200 g, had free access to a commercial pelleted diet (Oriental MF, Oriental Yeast Co. Ltd) and water. The animals were given an i.p. injection of pilocarpine hydrochloride (20 mg/kg, Wako Pure Chemical Industries, Ltd) dissolved in saline, after starvation for 16 h. Parotidectomy was performed under anesthesia with sodium pentobarbital (16 mg/kg, Abbot Laboratories) 30 min before the injection of pilocarpine. Animals were killed by a blow on the head 2 h after the administration of pilocarpine. The submandibular, sublingual and parotid glands and pancreas were dissected out, rinsed with saline, weighed and homogenized in saline using Polytron (Kinematika, GmbH) homogenizer. Amylase activity in the homogenates and serum was assayed photometrically using blue insoluble starch as substrate (Neo-Amylase Test, Daiichi Pure Chemical Co. Ltd). The method is based on measurement of the released blue dye from the substrate by the enzyme reaction.

Results and discussion. Table 1 shows the effects of pilocarpine on amylase activity in the submandibular, sublingual and parotid glands, pancreas and serum, Since in our preliminary experiments we concentrated on proving only that there was an increase in amylase activity in the submandibular gland after pilocarpine administration, there are more results for this gland than for other tissues. Amylase activity was decreased in the parotid gland (to 49%) and pancreas (to 45%) and was increased in the submandibular gland (to about 45-fold), sublingual gland (to about 23-fold) and serum (to about 7-fold) by the injection of pilocarpine, compared with the activity in the untreated control rats. We further investigated the possibility that the increase in amylase activity in the serum by the administration of pilocarpine might result from a passage of the enzyme produced in the parotid gland or pancreas into the blood. Table 2 shows amylase activity in parotidectomized rats. The activity in parotidectomized rats in-

Table 1. Amylase activity in various tissues of pilocarpine-treated rats

Tissue	Control rats (units)*	Pilocarpine-administered rats (units)*	Change in ratio of activity
Submandibular gland	0.0683 ± 0.0098(14)	3.0412 ± 0.3050(12)	44.52
Sublingual gland	0.3438 ± 0.0663(4)	7.9630 ± 0.9122(5)	23.16
Parotid gland	1373 ± 126(4)	671 ± 29(5)	0.49
Pancreas	381 ± 73(3)	173 ± 41(4)	0.45
Serum	0.1672 ± 0.0173(4)	1.1429 ± 0.0654(5)	6.84

Results are expressed as the mean ± SEM with the number of animals in parentheses. * 1 unit of amylase activity is defined as the amount of enzyme catalyzing the change in 1.0 of absorbance at 620 nm per 30 min per 1.0 mg of the tissue or 1.0 µl of the serum at 37 °C.

Table 2. Effect of pilocarpine administration on amylase activity in various tissues of parotidectomized rats

Tissue	Control rats (units)*	Pilocarpine-administered rats (units)*	Change in ratio of activity
Submandibular gland	0.1398 ± 0.0152(5)	0.1298 ± 0.0283(8)	0.93
Sublingual gland	0.7784 ± 0.0924(5)	0.9802 ± 0.2257(8)	1.26
Pancreas	358 ± 28(5)	204 ± 18(8)	0.57
Serum	0.2101 ± 0.0344(5)	0.2289 ± 0.0194(8)	1.09

All animals were parotidectomized under anesthesia with sodium pentobarbital 30 min before the injection of pilocarpine or saline. Results are expressed as the mean ± SEM with the number of animals in parentheses. * 1 unit of amylase activity is defined as the amount of enzyme catalyzing the change in 1.0 of absorbance at 620 nm per 30 min per 1.0 mg of the tissue or 1.0 µl of the serum at 37 °C.

creased about 2-fold in the submandibular and sublingual glands and increased slightly in the serum compared with the activity in unoperated rats. The activity did not, however, increase in the submandibular or sublingual glands or serum of the stimulated rats with pilocarpine. Amylase activity in the pancreas of the parotidectomized rats was almost the same as that of unoperated rats and decreased to 57% after the injection of pilocarpine. Stimulation by pilocarpine led to 51% and 55% depletions of amylase from the parotid gland and pancreas and a 7-fold increase in the activity in the serum of normal rats, respectively (table 1), but the depletion from the pancreas did not lead to an increase in serum amylase activity of parotidectomized rats (table 2). These results indicate that depletion of amylase from the parotid gland caused an increase in amylase activity in the serum and that the increase of serum amylase concomitantly produced considerable rises in amylase activity in the submandibular and sublingual glands. In the parotidectomized rats, sodium pentobarbital was used for anesthesia of the rats. However, sodium pentobarbital alone had no influence on the amylase activity in the salivary glands.

Shear et al.[6] reported that the rise in amylase activity in the rat submandibular gland after stimulation by pilocarpine was due to an increased activity of the enzyme in the blood vessels distributed around the glands. But they did not identify the origin of the increase of the enzyme activity in the blood. It is unlikely that the contamination of the blood causes the increase in amylase activity in the submandibular and sublingual glands because the activity was still high in these glands after perfusion with saline.

A primary step in stimulation of amylase release from the parotid gland by cholinergic agents is an increased influx of Ca^{2+} into the gland cells[2]. Driesbach reported that pilocarpine increased Ca^{2+} transport through membranes in vitro[7]. The administration of pilocarpine increased amylase activity in saliva (data not shown). These observations indicate that the stimulation by pilocarpine leads to a marked increase in amylase activity in saliva secreted through secretory ducts from the parotid gland. Our results suggest that pilocarpine may increase not only the secretion of amylase into the secretory ducts but also permeability of the enzyme into the blood stream. We are unable to demonstrate how amylase in the blood is taken up into the submandibular and sublingual glands. The mechanism of uptake of amylase from the blood into the salivary glands remains to be further elucidated.

1 A.P. Vreugdenhil and P.A. Roukema, Biochim. biophys. Acta *413*, 79 (1975).
2 E.L. Watson, J.A. Williams and I.A. Siegel, Am. J. Physiol. *236*, 233 (1979).
3 L.H. Schneyer and C.A. Schneyer, N.Y. Acad. Sci. *85*, 189 (1960).
4 A.H. Kuijper-Lenstra, M.F. Kramer and W.J. van Venrooij, Cell Tissue Res. *164*, 447 (1975).
5 C.F. Chignell, Biochem. Pharmac. *17*, 2225 (1968).
6 M. Shear, S. Gibson and E. Merwe, J. Histochem. Cytochem. *21*, 661 (1973).
7 R.H. Driesbach, Am. J. Physiol. *204*, 497 (1963).

Vitellogenin and lipovitellins in *Orchestia gammarellus* (Pallas) (Crustacea, Amphipoda); labelling of subunits after in vivo administration of ^{3}H-leucine

Henriette Junéra and J.-J. Meusy

Laboratoire de Sexualité et Reproduction des Invertébrés, E.R.A. No. 409, Université Pierre et Marie Curie, Bâtiment A, 4 place Jussieu, F-75230 Paris Cedex 05 (France), 30 January 1981

Summary. The distribution of ^{3}H-leucine between the various polypeptide components of *Orchestia gammarellus* vitellogenin and lipovitellins, separated by SDS-PAGE, has been studied after in vivo injection of this amino-acid. The results corroborate the view that the heaviest components, or native polypeptide subunits of vitellogenin, are progressively transformed into lighter ones.

In the amphipod *Orchestia gammarellus* (Crustacea, Amphipoda) vitellogenin (VTG), the precursor of vitellus synthesized by the sub-epidermal adipose tissue[1,2], lipovitellin I (LPV I), the main component of that vitellus, and lipovitellin II (LPV II) were impossible to differentiate on the basis of polyacrylamide gel electrophoresis (PAGE) and immunological studies[3].

The question arises whether the VTG undergoes any chemical modification during vitellogenesis. Electrophoresis performed in a denaturing medium has revealed differences in the composition of VTG and both LPVs[4]. VTG is composed of a large number of components (8–11), the molecular weights of which range from 31,000 to 235,000. In the course of vitellogenesis, during which VTG is transformed into LPV I and LPV II, there is a gradual disappearance of the largest subunits (mol.wt > 127,000). When egg-laying occurs, those with a mol.wt above 200,000 are totally absent from the lipovitellins[5].

We have postulated that VTG is synthesized in a form which only includes 2 components with high molecular weights, i.e. 217,000 ± 17,000 and 235,000 ± 10,000. These *sensu stricto* subunits would then undergo a process of proteolytic cleavage starting even before the entrance of VTG into the oocytes. Analogous phenomena seem to occur in certain insects[6,7].

In the present paper, the process was studied by following the distribution of label among the various components of VTG and the LPVs 3, 6 and 20 h after injection of tritiated leucine.

3 groups of animals were formed, each including 30–65 females in vitellogenesis. Each female was first injected with 2.5 µCi tritiated leucine, and samples of a few µl of hemolymph were taken after 3 h (1st group), 6 h (2nd group) and 20 h (3rd group). The specific activity of the tritiated leucine injected was 48–50 Ci/mM for the 1st 2 groups, and 146 Ci/mM for the 3rd (L-(4–5 ^{3}H) leucine, Radio-Chemical Centre, Amersham, England).

The VTG and LPVs were isolated by preliminary PAGE followed by electrodialysis, and were then submitted to SDS-PAGE according to previously described techniques[4].