# Effects of Removal of Superior Cervical Ganglion or Auriculotemporal Nerve on Course of Postnatal Change in Rat Parotid Amylase (36578)

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Amylase activity of rat parotid gland has been shown to increase progressively during postnatal development (1–3), reaching a plateau within 7–8 weeks (4). An intact autonomic innervation to the gland has been shown to be necessary for expression of the normal pattern of postnatal change (4). The separate role of the sympathetic and parasympathetic branches on these changes has not however been delineated. The present investigation was therefore undertaken to determine the individual effects of the two autonomic pathways.

*Materials and Methods.* Long-Evans rats, ranging in age from 8 days to 3 months, were used in these experiments. After weaning, animals were maintained on lab chow and water *ad libitum*, and maintained in temperature-controlled quarters which were lighted from 7 a.m. to 7 p.m. and darkened from 7 p.m. to 7 a.m. At 8 days of age, under light ether anesthesia, the superior cervical ganglion was unilaterally removed from litter mates; at the same time, part of the auriculotemporal nerve was unilaterally removed from other rats of the same litters; in addition, some animals were subjected to sham operations, while others remained as unoperated controls. At selected times after the denervation (16, 23, 32, 49, 64 and 86 days), the paired unstimulated parotid glands from these animals were removed, under Nembutal anesthesia (50 mg/kg, ip), each was rapidly weighed on a torsion balance and then placed in a freezer (−15°) for subsequent amylase determination, or immediately homogenized at 0–4° with 0.4 *N* $HClO_4$ for extraction of nucleic acids. With some groups of animals, a period of food deprivation (unfasted) did not precede the chemical determinations; in others (litter mates of the unfasted rats), food but not water was removed from rats at either 5 or 10 p.m. and animals were sacrificed the next morning between 9–10 a.m. (fasted rats). If rats were younger than 23 days of age, they were deprived of food for no more than 12 hr. Amylase activity of the glands was determined by the method of Myers, Free and Rosinski (5), using properly diluted samples of the supernatant material from the gland, and was expressed as milligrams of reducing substance (as glucose) formed during a 15-min digestion period per milligram of wet tissue, or per microgram of DNA, or per gland. Nucleic acids were extracted from the whole glands and the amount was determined by methods described by Schneider (6). The whole gland was homogenized at 0–4° in 0.4 *N* $HClO_4$, and then centrifuged. The supernatant fluid which contains acid-soluble nucleotides (including ATP) was discarded, and the precipitate then was dispersed, washed 3 times with cold $HClO_4$, and then hydrolyzed at 90° for 15 min in 0.4 *N* $HClO_4$. Total DNA was determined using the diphenylamine reaction (7).

*Results.* Removal of the superior cervical ganglion (Sx) or part of the auriculotemporal nerve (Px) at 8 days of age resulted in marked modification in the course of postnatal changes in amylase concentration (amylase activity per milligram of wet weight or microgram of DNA) or total amylase activity of parotid glands from rats deprived of food for 12–18 hr prior to enzyme determi-

[1] Supported in part by Grant DE 02110 from National Institutes of Health.

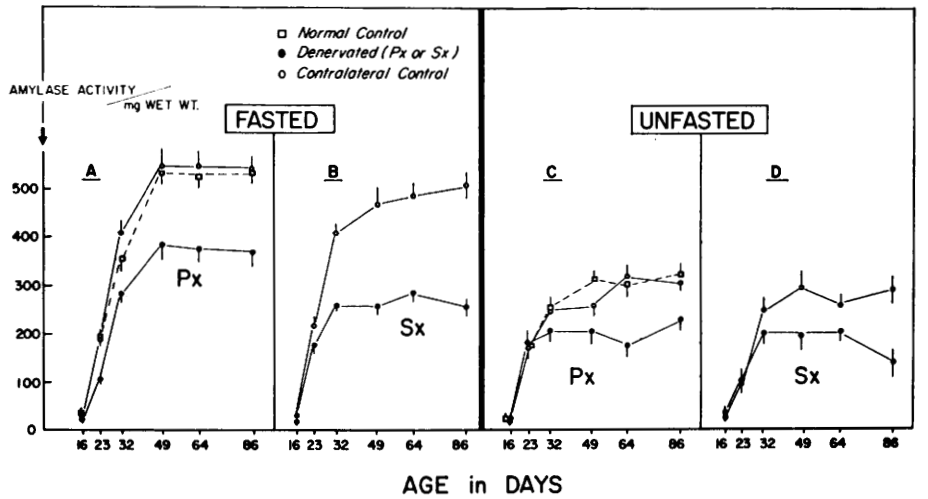


FIG. 1. Time course of postnatal changes in amylase activity per milligram of wet weight of denervated (●) parotid glands (removal of superior cervical ganglion, Sx; or removal of part of auriculotemporal nerve, Px); normally innervated contralateral glands [contralateral control (○)] and normally innervated glands of unoperated rats [normal control (□)], fasted and unfasted. Each point represents the mean of values from no more than 10 rats, and no less than 5, and the perpendicular bars indicate the SE of the means.

nation. Although neither of the denervations prevented the progressive increase with age observed in innervated glands, the levels reached were, at all except 16 days of age, considerably less than those of the contralateral innervated glands or glands of unoperated rats. With Sx glands, amylase activity (per mg wet weight, or per $\mu$g DNA) reached maximal levels (250 and 45 mg, respectively) by 32 days of age, and remained at this level thereafter (Figs. 1 and 2). Plateau levels (450 mg/mg, and 102 mg/$\mu$g DNA) were not established for the innervated gland until 49 days of age. At all ages after 23 days of age, the concentration of amylase in the Sx gland was approximately one half that of the innervated mate (Figs. 1 and 2).

Total amylase activity of the Sx glands as well as that of innervated glands continued to increase to at least 7–8 weeks of age (Table I). Present data (Table I) as well as previously published values (8–10) show that total DNA and wet weight of Sx and innervated glands also increase progressively for 7–8 weeks postnatally. However, at no age did DNA of the Sx gland differ from that of the innervated mate, and total weight of the Sx gland was the same or only 10–12% less than that of the innervated member of the pair at all ages (Table I). On the other hand, at each age after 23 days of age, total amylase of the Sx glands was only one half that of the innervated member of the pair (Table I). Thus, the 50% reduction in amylase effected by sympathectomy is essentially a reflection of a 50% reduction in amylase activity/cell.

Significant differences ($p < .01$) between amylase levels of parasympathectomized and normally innervated glands were observed at 23 days of age, and total amylase activity as

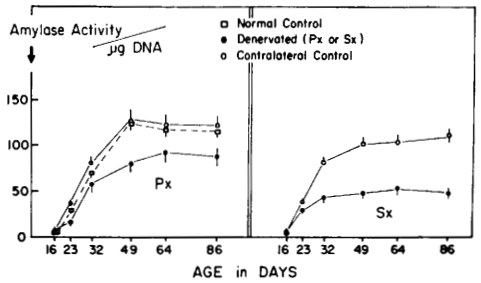


FIG. 2. Time course of postnatal changes in amylase activity per microgram of DNA of denervated and innervated glands of fasted rats. See Fig. 1 for details. {DNA values mainly taken from previously published values [Schneyer and Hall (10)]}.

TABLE I. Effects of Selective Postganglionic Denervation on Time Course of Postnatal Changes in Total Amylase Activity of Parotid Gland of Fasted Rats.

| Age (days) | No. of rats | | Total amylase activity[b] | | | | Wet wt (mg) (% reduction)[d] | | DNA (μg)[c] (% reduction)[d] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Px | Sx | Px | Con | Sx | Con | Px | Sx | Px | Sx |
| 16 | 6 | 5 | 418 ± 76 | 411 ± 59[e] | 345 ± 64 | 207 ± 42 | 19 | 0 | 16 | 0 |
| 23 | 6 | 5 | 3654 ± 348 | 11,083 ± 400 | 9324 ± 753 | 12,712 ± 443 | 30 | 0 | 22 | 0 |
| 32 | 7 | 8 | 15,763 ± 1521 | 34,522 ± 2600 | 17,400 ± 800 | 35,498 ± 2400 | 41 | 12 | 34 | 0 |
| 49 | 9 | 9 | 31,000 ± 3000 | 75,556 ± 600 | 25,190 ± 1835 | 56,829 ± 789 | 48 | 10 | 34 | 0 |
| 64 | 5 | 7 | 36,000 ± 1076 | 83,000 ± 1600 | 38,850 ± 359 | 75,629 ± 460 | 44 | 11 | 43 | 0 |
| 86 | 5 | 5 | 26,101 ± 3545 | 82,000 ± 4700 | 28,000 ± 521 | 86,000 ± 955 | 46 | 10 | 46 | 0 |

[a] Values are means ± SE. Value of contralateral control (Con) is, at each age, significantly different from denervated gland (Px = removal of auriculotemporal nerve, or Sx = removal of superior cervical ganglion) ($p < .01$), except where indicated by *e*. Differences between amylase values for successive time intervals are statistically significant ($p < .01$) for denervated (Px or Sx) and innervated glands (Con) of fasted as well as unfasted rats, until 49 days of age.

[b] Amylase activity is expressed as milligrams of reducing substance (as glucose) formed per 15 min/gland.

[c] Values in these columns calculated from data in Ref. (10), and additional new data; 5–10 rats used for each value.

[d] Percentage reduction from innervated contralateral gland.

well as activity per unit of wet tissue or DNA of Px glands were nearly 50% less than levels of innervated mates (Figs. 1 and 2 and Table I). By 32 days of age, the differences in concentration between denervated and innervated glands was only 30% and the magnitude of reduction remained essentially at this level at all intervals thereafter. In contrast to Sx glands, plateau concentrations were higher (370 mg/mg wet wt and 85 mg/$\mu$g DNA) and were not reached until 49 days of age. Total amylase activity, total DNA and wet weight also reached maximal levels by 7–8 weeks of age. While total amylase activity of Px glands was usually less than one half that of innervated glands, total DNA and wet weight were usually reduced by about 35%. With parasympathectomy there is also a reduction in amylase per cell but the magnitude of the change in cell levels of amylase was much less than that observed with sympathectomy. Since total DNA and weight of the Px glands are reduced by approximately 25-35%, it is clear that the reduction in total amylase is a reflection of these changes as well as a decrease per cell.

If animals were not fasted prior to amylase determination, progressive increases in amylase activity per milligram wet weight, or in total amylase activity with increasing age (Table I) were still observed with increasing age in Sx, Px and normally innervated glands. However, maximal levels reached were, in each case, distinctly lower than those of fasted animals, and in fact, levels of Sx glands could not be distinguished from those of Px glands. Furthermore, although differences between the levels of denervated and innervated glands were evident, these differences were small and frequently statistically insignificant ($p > .01$; Fig. 1 and Table I).

*Discussion.* Previously it was shown that surgical removal of both branches of the autonomic innervation to rat parotid at 8 days of age did not prevent the progressive increases in amylase activity of the gland but did markedly modify the course and extent of these changes (4). These effects were attributed to the resulting diminution in autonomically mediated glandular activity that occurred as a consequence of the denervation. The present study shows that the course of postnatal change can be modified by removal of either branch of the innervation and that the specific alterations induced depend on which branch is removed. This specificity of neural regulation has also been shown with regard to regulation of development changes in size of the gland, and secretion of electrolytes (1, 10), and is therefore not an unexpected finding. It is however surprising that the effects of sympathetic denervation are in some respects more striking than those induced by parasympathectomy. For example, the present work clearly shows that sympathectomy results in more pronounced effects on levels of amylase per unit of tissue than does parasympathectomy. On the other hand, the effects on total amylase activity of the gland under the two conditions of denervation do not differ from each other. However, the bases for the reductions in total amylase under the two conditions of denervation are very different. With sympathectomy, gland mass is not appreciably changed: cell number of the Sx gland does not differ from, and gland size is at most only 10–12% less than that of the innervated gland, at any age; on the other hand, gland mass is markedly altered by parasympathectomy, and both cell number and gland size of parasympathectomized glands show reductions from control levels that amount to as much as 50% (10). While reduction in amylase activity per cell is found with either kind of denervation, the reductions induced by sympathectomy amount to as much as 50% whereas with parasympathectomy, they are 30%. Since gland mass is essentially unchanged with sympathectomy, it is clear that the marked reductions in total amylase of sympathectomized glands reflect almost exclusively the concomitant reduction in amylase per cell; with parasympathectomy, on the other hand, reduction in gland mass accounts for most of the decrease in gland amylase, and reductions in amount per cell may account for only a small fraction of the changes.

The present data further emphasize the importance of a period of fasting before enzyme determination (4). Since the parotid gland exhibits a definite secretory cycle, maximal amylase levels are observed only when fasting precedes amylase estimation. Thus, it is not unexpected that the normally inner-

vated glands of fasted rats show much higher amylase levels than do glands of unfasted rats. It is also apparent that in order to establish the role of the separate branches of the innervation in regulation of amylase, a period of fasting is a necessary prerequisite. Without the period of fasting, the levels under the two conditions of denervation are very similar; with fasting, the levels of the parasympathectomized are much higher than those of the sympathectomized glands. Since it can be assumed that, with fasting, the remaining intact branch is also relatively inactive, the levels observed under these conditions must reflect most accurately the role exerted by each branch of the innervation in regulation of amylase. In the unfasted state, it may be assumed that the remaining intact branch induces glandular activity and causes depletion of gland stores of amylase. Thus, with sympathetic fibers removed and only parasympathetic fibers active, only a modest depletion of gland amylase is effected (as judged from the difference between levels of fasted and unfasted Sx glands). This is not unexpected since cholinergic stimulation does not cause marked secretion of amylase nor marked depletion of gland stores (11, 12). On the other hand, with parasympathetic fibers removed, only sympathetic fibers are active and the depletion induced by sympathetic activity is more marked than that observed with parasympathetic activity (13). These findings are consistent with the observed effects of parasympathetic and sympathetic nerve stimulation on secretion of amylase from salivary glands (14).

The effects on amylase levels of a modified glandular blood flow have not been ascertained. It is possible that as a consequence of the denervation, blood flow is sufficiently altered so that protein synthesis is affected. Effects of such changes are being examined.

*Summary.* Elimination of the postganglionic fibers from either branch of the autonomic nervous system, at 8 days of age, markedly modifies the course of postnatal change in amylase levels of rat parotid gland. Total gland amylase of parasympathectomized glands is, at all ages after 16 days, only 1/3 to 1/2 that of the innervated member of the pair; amylase activity per unit of wet weight or DNA was also lower. Since gland size and cell number are also concomitantly modified by the parasympathectomy, the reduction in total amylase activity of parotid appears to be chiefly a reflection of the decrease in gland mass; and the reduced activity per cell accounts for only a small portion of the decrease. On the other hand, removal of the superior cervical ganglion resulted in decrease of 50% in concentration as well as total amount per gland. Furthermore, since neither cell number (no change) nor gland size (10–12% change) of the sympathectomized gland is different from that of the innervated gland, the decrease in total amount of amylase was here chiefly attributed to the decrease per cell. Clarification of the separate roles of the two branches of the innervation depended on establishment of maximal gland levels of amylase; this was accomplished by a period of fasting prior to enzyme determination.

The authors thank Mrs. Jean Otwell for competent technical assistance.

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Received Mar. 15, 1972. P.S.E.B.M., 1972, Vol. 140.