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Premature Increases in Amylase of Postnatal Rat Parotid with Chronic Isoproterenol[1] (39825)

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Introduction. Rat parotid gland, like submaxillary (1), is incompletely developed at birth, and morphological and functional differentiation continue for some time postnatally (2). Morphological changes include increases in cell size and number, and differentiation of presumptive acinar cells into mature acinar cells (3). Functional changes include progressive increase in levels of the digestive enzyme, amylase (4-6), and alteration in the ratio of Na/K secreted in the saliva (7). Chronic administration of isoproterenol has been shown to alter the course of postnatal morphological changes (8), with specific changes dependent on the period during development when isoproterenol is given (9, 10). In general, the effects include an increase in cell size at all stages of postnatal development, but an increase in proliferative activity only at postweanling stages; at earlier stages, isoproterenol suppresses mitosis (9) but accelerates the differentiation of presumptive acinar cells into mature acinar cells (8, 11).

The effects of chronic administration of isoproterenol on secretory function have not been delineated. Since the morphological effects include a marked acceleration of differentiation of acinar cells, the object of the present investigation was to assess a functional aspect of development associated almost exclusively with such cells. In rat parotid, the digestive enzyme amylase is synthesized and stored in acinar cells only (12, 13). Accordingly, the effects of chronic administration of isoproterenol on developmental changes in parotid amylase levels have been investigated. Since isoproterenol affects the size and mitotic activity of developing acinar cells, the change in cellular amylase level as well as that in the entire gland were also examined.

Materials and methods. Long-Evans rats, 8-32 days of age, were used in these experiments and, after weaning, were maintained on lab chow and water ad libitum. DL-Isoproterenol bitartrate (ISO) in physiological saline was injected ip twice daily in an average dose of 13 mg/kg to neonatal rats 9 or 23 days of age at the time of initial injection of the drug. Only one-half of each litter was injected with equal volumes of saline (0.05-0.15 ml). The ISO or saline regimen was terminated at 18 or 36 hr before gland removal, and, from some groups, food (but not water) was removed 12-18 hr prior to gland removal; with other groups, food was not removed prior to gland removal. Under Nembutal (1%) anesthesia, parotid glands were excised and rapidly weighed on a torsion balance. Part of one gland was then placed in Bouin's for subsequent histological examination; the other portion was weighed, and placed in a freezer (−15°) for subsequent amylase determination. The other member of the pair was placed in ice-cold 0.4 *N* $HClO_4$, homogenized, and then centrifuged. The supernatant fluid was discarded, and the precipitate was then dispersed, washed three times with cold $HClO_4$, and then hydrolyzed at 90° for 15 min in 0.4 *N* $HClO_4$. Nucleic acids were extracted from the whole glands and amounts were determined by methods described by Schneider (14). Total DNA was determined using the diphenylamine reaction (15); total RNA was determined using the orcinol reaction (16), or, since the two methods agreed well, by subtracting DNA from total nucleic acids measured spectrophotometrically at 260 μm. Amylase activity was determined by the methods of Myers *et al.* (17), using properly diluted samples of the supernatant material from the gland homogenate, and was expressed as milligrams of reducing substance (as glucose) formed during the 15-min digestion period per milli-

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gram of wet gland.

Optimal conditions for establishment of maximal amylase levels in glands of postnatal rats (untreated as well as those chronically treated with isoproterenol) were determined. Fasting of rats prior to enzyme assay was not necessary for preweanling rats, but was for postweanling animals; nonetheless, for consistency of treatment, all postnatal age groups were fasted for an appropriate length of time (12 hr for 19-day-old rats; 18 hr for older rats) prior to gland removal. It was also found that to establish maximal amylase levels in isoproterenol-treated animals, a 30-hr interval between last injection of isoproterenol and gland removal was necessary.

Results. Chronic administration of large doses of isoproterenol to neonatal rats caused marked changes in weight, total DNA, RNA, and amylase activity of parotid gland. The magnitude and direction of the changes, however, depended on the animal's age when the drug regimen was initiated and terminated. Thus, if animals were 9 days of age at the time of the initial administration of isoproterenol, and 17 days of age at the time of the last injection, total parotid DNA of the 19-day-old ISO-treated rats was 28% less than that of control glands ($P < 0.05$), whereas values for weight, total RNA, and total amylase of parotid of the ISO-injected animals were about two times greater than those of litter-mate controls (Table I). A 12-hr period of fasting prior to amylase determination did not cause modification in amylase levels in these 19-day-old animals, and levels of fasted and unfasted controls were approximately 3500 and 3300 mg, respectively (Table I).

Since weight and nucleic acids were affected differently by the isoproterenol regimen, amylase activity in relation to each of these parameters was assessed to obtain estimates of amylase per unit of tissue or cell. Thus, concentration of amylase based on activity per milligram wet weight of tissue or per microgram of RNA was, for parotid of ISO-treated 19-day-old rats, not different from that of controls ($P < 0.05$). However, when concentration of amylase was based on amylase activity per microgram of DNA, there was a marked difference between glands of isoproterenol-treated and control animals, and amylase activity per microgram of DNA of ISO-treated rats was nearly three times greater than that of controls (Table I).

If animals were past weaning (23 days of age) when the isoproterenol regimen was initiated, the changes induced were again marked, but the magnitude and direction of the changes differed sharply from those observed in the preweanling groups. When glands were removed at 32 days of age, total weight and RNA of parotid of ISO-treated rats were about four times greater than those of control glands, and DNA was increased by nearly 60% (Table I). However, total amylase of the ISO-treated gland was

TABLE I. AMYLASE ACTIVITY AND NUCLEIC ACID CONTENT OF PAROTID OF PREWEANLING AND POSTWEANLING RATS FOLLOWING CHRONIC ISOPROTERENOL.

Condition[a]		Age of sacrifice (days)	No. of days on ISO	PA wt (mg)	Total DNA[b] (μg/gland)	Total RNA[b] (μg/gland)	Amylase Activity: Total (mg/gland)	Amylase Activity: Concentration[c] mg/mg wet wt	mg/μg DNA	mg/μg RNA
Nonfasted, 30	(6)	19	0	33 ± 1[d]			3276 ± 110	122 ± 3	12	12
Fasted, 30	(6)	19	0	32 ± 3	281 ± 9	265 ± 12	3537 ± 389	105 ± 10	13	13
Fasted, 30	(6)	19	8	57 ± 5*	201 ± 9*	572 ± 68*	6160 ± 711*	97 ± 7	31	11
Nonfasted, 30	(6)	32	0	117 ± 6			29,863 ± 2235*	264 ± 33	56	20
Fasted, 30	(13)	32	0	98 ± 2	536 ± 37	1462 ± 67	43,508 ± 2509	463 ± 76	81	30
Fasted, 30	(13)	32	8	427 ± 6*	851 ± 31*	6278 ± 224*	62,294 ± 2406*	148 ± 7*	73	10
Fasted, 18	(5)	32	8	507 ± 39*			38,240 ± 2635	76 ± 2*	45	6

[a] Food was removed from 19-day-old rats 12 hr before sacrifice, and from 32-day-old rats 18 hr before sacrifice; number following condition indicates interval of time (in hours) elapsing between last injection of ISO or saline and sacrifice; numbers in parentheses refer to number of rats.

[b] Previously reported. Also, DNA and RNA of fasted rats do not differ significantly from levels of unfasted ($P < 0.05$) (6).

[c] Concentration of amylase is expressed as follows: (total gland amylase/total gland weight, or total DNA, or total RNA) amylase activity expressed as milligrams of reducing substance per milligram wet weight of gland, or per microgram DNA of gland, or per microgram RNA of gland.

[d] Values are means ± SE.

* Differs significantly from fasted controls ($P < 0.05$).

about 50% greater than that of controls (Table I). This difference in amylase was recorded when food had been withdrawn for 18 hr prior to gland removal. When there was only an 18-hr interval between the last injection of the drug and gland removal, amylase levels did not differ from those of the controls.

Although total parotid amylase of the ISO-treated rats was higher than that of controls, concentration of amylase, based on activity per milligram wet weight of tissue, or per microgram of RNA, was higher in the controls than it was in glands of ISO-treated animals. In fasted rats, the concentration (either per milligram wet weight or per microgram of RNA) of control glands was three times as great as that of the ISO-treated glands (where last ISO injection was 30 hr before gland removal). When amylase concentration was expressed as activity per microgram of DNA, however, there was little or no difference between controls and ISO-treated rats (Table I).

Histological evidence of changes in cell size, mitotic rate, and degree of cellular differentiation were also obtained for correlation with the biochemical parameters. The isoproterenol regimen, as reported earlier (8), has a conspicuous effect on acinar or acinar-like cells, but no discernible influence on ducts. Thus, at 19 days of age, cell size of the ISO-treated parotid is increased twofold from that of controls; and the change from an undifferentiated proacinar cell to a differentiated acinar cell is apparent. In 32-day-old rats, the increase in acinar cell size is much greater, and acinar cells of ISO-treated animals are three or four times as large as those of controls; in this age group, however, acinar cells of controls are fully differentiated. Mitotic figures are numerous in the 19-day-old control, but few are evident in the ISO-treated gland. Conversely, in the 32-day-old rat, there are more mitotic figures in the ISO-treated than in the control gland. Nuclear size of the ISO-treated glands did not differ from that of controls, in either the 19- or 32-day-old age group.

Discussion. A progressive increase in amylase levels of rat parotid occurs during postnatal development (4–6). Present data show that the levels are prematurely increased when isoproterenol is chronically administered to the neonates. The magnitude of the increases, and the cellular basis for them, depend on the postnatal period during which the drug is administered. When the isoproterenol is administered to the preweanling rat, total amylase is double that of litter-mate controls, amylase per unit of cytoplasm (based either on amount per milligram wet weight or per microgram of RNA) of the ISO-treated and control glands is the same, and amylase per microgram of RNA of the ISO-treated parotid is nearly three times that of controls. Total mass of the ISO-treated gland is also double that of controls, but total DNA is even less than control levels. From these facts, it is evident that the increase in total amylase can be attributed solely to the cellular hypertrophy induced by the isoproterenol and not to increased cell number, since the increase in total amylase just corresponds to the increase in total cytoplasmic mass, and cell number is not increased. The ability of the tissue to produce amylase proceeds apace with the increase in cytoplasmic volume.

When the isoproterenol is administered to the postweanling rat, where mature acini are abundantly evident, total amylase levels are only 50% greater than those of controls, but gland mass is four to five times greater (based on wet weight as well as RNA). However, DNA content is also about 50% greater than controls and, since the amylase per unit wet weight or per microgram of RNA is only one-third that of control levels, and amylase per microgram of DNA is not different from control levels, it may be concluded that production does not keep pace with the increased cytoplasmic volume and that the increase in total amount of amylase must be attributed solely to the increased amount of DNA (or number of cells). The fact that the increases in these two (DNA and amylase) are, percentage-wise, very similar (about 50%) lends further support to this view.

Since progressive increase in amylase level of parotid is part of the normal maturation process in immature rat, the precocious elevation of total parotid amylase in both preweanling and postweanling rats to levels

found normally at a more advanced age (6) can be interpreted as acceleration of amylase development. In the preweanling rat there is little doubt that the increase in total amylase of the ISO-treated gland can be interpreted as acceleration of functional development; first, the increase in total amount of amylase corresponds to the increase in total protoplasmic volume; second, such increases are related only to increased cytoplasmic volume since DNA of the ISO-treated glands is in fact less than that of the controls; third, the fact that amylase per microgram of RNA of ISO-treated and control glands is the same indicates that each unit of protoplasm is producing the usual amount of amylase but that more units of cytoplasm are producing the enzyme. The increase in the postweanling ISO-treated rat must be somewhat more cautiously interpreted as evidence of accelerated development. The increase in total amylase in these animals is related to increased DNA; the same is true in adult ISO-treated glands (18, 19). However, percentagewise, the increase in total DNA and total amylase (in each case, about 50%) in the ISO-treated postweanling rat is greater than that found in parotids of ISO-treated adults, where the increase in total amylase is only about 15%, but the increases in total DNA may be considerably higher (18, 19). Thus, acceleration of amylase accumulation probably occurs also in the postweanling rat, but the cellular basis for the increase is different from that of the preweanling animals. Indeed, the differences in response of the early postweanling and adult glands may be related to the fact that polyploidy in adult ISO-treated glands has been definitely established (20, 21), but while some data suggest that this occurs in submaxillary glands of immature rats treated with isoproterenol (22), from preliminary present data on measurements of nuclear size of control and ISO-treated parotid glands of immature rats, this cannot yet be concluded.

These findings also provide additional evidence of neural regulation of developmental changes in amylase levels of rat parotid. It has been shown that, in the absence of the sympathetic or parasympathetic pathway, the normal postnatal developmental increases in amylase of the gland or cell are inhibited (6). In this regard the role of the sympathetic is especially pertinent to present conclusions, since the levels per unit of DNA are decreased as a consequence of sympathectomy (but not of parasympathectomy). In the present work, data show that the premature development caused by the β-adrenergic agent isoproterenol results in an increase in amylase per unit of DNA. Thus, a decrease or increase in amylase depends on a decrease or increase in adrenergically mediated glandular activity.

Finally, the extent of normal neural mediation of glandular activity may play a decisive role in regulating the magnitude of growth responses to isoproterenol. Thus, in the postweanling rat, normal neural influences on growth are prominent; these effects may be superimposed on those induced by the isoproterenol, whereas, in the preweanling, no conspicuous neural influences are normally seen. Such differences in normal physiological mediation of growth may account for differences in magnitude of, e.g., the hypertrophic response of postweanling (fourfold) and preweanling (twofold) parotid glands. The relation to normal growth characteristics thus may be secondary to these events.

Summary. Chronic administration of large doses of isoproterenol during postnatal development caused an increase in amylase levels of parotid gland of the immature rat. The magnitude of the increase and the cellular basis for amylase changes depended on the postnatal period during which isoproterenol was given. If given during a preweanling period (9–17 days of age), total amylase as well as gland and cell size and total RNA were twice the levels of control litter-mates; the amount of amylase per unit of cytoplasm or per microgram of RNA was unchanged from levels of controls but the amount per microgram of DNA was nearly three times that of controls. If given during a postweanling period (23–31 days of age), the increase in total amylase corresponded to the increase in total DNA (about 50% increase in each when compared with controls), but the amount per unit of DNA was unchanged from controls, and the amount per milligram tissue wet weight or micro-

gram of RNA was in fact only one-third the control levels. In the preweanling rat, the ability of the parotid to produce amylase keeps pace with the increase in cytoplasm in the preweanling rat but not in the postweanling rat. The increases in amylase in the preweanling gland are interpreted as evidence of an acceleration of functional development (increased accumulation of amylase), but in postweanling rats the increases in amylase do not provide unequivocal evidence for accelerated development.

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