A Growth-Suppressive Influence of L-Isoproterenol on Postnatally Developing Parotid Gland of Rat[1] (37437)

CHARLOTTE A. SCHNEYER

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

Fully differentiated parotid and submaxillary glands of adult rat (more than 2 months of age) normally exhibit a low level of mitosis (1) but marked hyperplasia, and hypertrophy, can be induced in these glands by chronic stimulation with DL-, or L-isoproterenol (2, 3). The incompletely differentiated parotid and submaxillary glands of immature rats normally exhibit a high rate of mitosis (4, 5) and when treated chronically with DL-isoproterenol show accelerated morphological (6) and biochemical (6, 7) differentiation. While this agent also causes hypertrophy in parotid and submaxillary glands of immature rats (6), its ability to induce hyperplasia in such glands has not been ascertained. However, the effects of isoproterenol on the normally dividing gland cells of immature rats may be quite different from its effects on the mitotically stable glands of adults. In fact, the acceleration of development may occur as a consequence of a suppression of normal mitotic activity by isoproterenol. Since the developing gland exhibits two distinct phases of growth, an early one characterized by extensive proliferative activity and differentiation, and a post-weaning phase characterized by increase in cell and gland size, and reduced mitotic activity (5), examination of the effects of chronic administration of isoproterenol was made on mitotic activity during these two phases.

Materials and Methods. Long–Evans male and female rats, 2–38 days of age were used in these experiments. Post-weanling rats were maintained on lab chow and water *ad libitum*. Isoproterenol (ISO) or propranolol (Inderal) was injected ip twice daily in an average dose of 13 mg/kg. Isoproterenol was used as the L or D bitartrate salt. L-Isoproterenol was used rather than the DL-isoproterenol since the L-ISO has been shown to be the active component of the racemate in effecting growth changes in salivary glands (3, 8). The ISO or propranolol regimen was terminated 18 hr before removal of glands. Control animals were injected with equal volumes (0.05–0.15 ml) of saline or were untreated. Under Nembutal (1%) anesthesia, parotid glands were excised and rapidly weighed on a torsion balance. One gland was then placed in Bouin's solution for subsequent histological preparation and determination of mitotic counts and cell size (5). The other member of the pair was placed in ice-cold 0.4 N $HClO_4$ for extraction of nucleic acids (9). Determinations of DNA and RNA were made as previously described (5, 10).

Results. Chronic administration of L-isoproterenol (ISO) to rats 2–38 days of age modified the normal pattern of growth of parotid gland. When L-ISO was administered for a period of 8 days, parotid weight and total RNA of the gland, at each age examined, were always significantly higher than weight and RNA of glands in untreated litter mates (Table I). The magnitude of the increase was related to the age of the animals. Thus, although the same dosage and duration of treatment were used for all groups, if rats were only 11–13 days of age at the beginning of ISO administration, parotid weight showed less than a doubling after 8 days of drug administration; on the other hand, if animals were 21–29 days of age when ISO was begun, gland weight showed a four- to fivefold increase over controls. The

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TABLE I. Effects of Chronic Administration of D and L Isomers of Isoproterenol on Weight, DNA and RNA of Rat Parotid During Postnatal Development.[a]

Age at initial injection[b] (days)	Age at sacrifice (days)	No. days on ISO	Total parotid				
				DNA		RNA	
			Wet wt (mg)	(μg/gland)	(μg/mg)[c]	(μg/gland)	(μg/mg)[c]
11	19	8 (l)	56 ± 4 (6)	203 ± 9	3.6	572 ± 68	10.2
		8 (d)	44 ± 7 (6)	264 ± 42	6.0	378 ± 58	8.6
		0	32 ± 1 (6)	281 ± 9	8.8	266 ± 12	8.3
13	21	8 (l)	105 ± 7 (5)	302 ± 12	2.9	1368 ± 96	13.2
		0	65 ± 2 (5)	433 ± 19	6.7	686 ± 26	10.6
15	23	8 (l)	173 ± 12 (4)	533 ± 15	3.1	2466 ± 93	14.3*
		8 (d)	68 ± 3 (4)	421 ± 98	6.2	1008 ± 153	14.8
		0	56 ± 3 (9)	407 ± 10	7.3	900 ± 40	16.0
21	29	8 (l)	358 ± 15 (5)	822 ± 42	2.3	5305 ± 166	14.8*
		0	101 ± 4 (5)	535 ± 22	5.3	1456 ± 67	14.4
24	32	8 (l)	474 ± 22 (6)	851 ± 31	1.8	6278 ± 224	13.2*
		8 (d)	105 ± 4 (6)	583 ± 20	5.6	1425 ± 68	13.6
		0	117 ± 6 (6)	572 ± 19	4.9	1487 ± 119	12.7
29	36	7 (l)	343 ± 29 (3)	728 ± 57	2.1	4821 ± 324	14.1*
		7 (d)	114 ± 7 (3)	508 ± 13	4.5	1638 ± 57	14.4
		0	110 ± 5 (9)	527 ± 20	4.8	1452 ± 67	13.2

[a] Values are means ± SE. No. in parentheses refers to number of rats. L- or D-isoproterenol (ISO) in an average dosage of 13 mg/kg was injected twice daily for the number of days indicated in each case; (L) or (D), adjacent to No. of days, indicates form of isomer used. L-ISO values were significantly different from values of untreated rats in all casse ($p < 0.01$), except where indicated by *; D-ISO values were in no instance significantly different from values of untreated rats ($p > 0.01$).

[b] L or D ISO, or saline alone, was in each case injected.

[c] Milligrams wet weight of parotid gland.

changes in total RNA of glands in the older age groups paralleled the weight changes, but in the 11–13-day-old groups, this was not the case. Thus, in the younger age groups the ratio, micrograms RNA/milligram wet weight of gland, was higher for L-ISO-treated rats than for controls; in the older age groups these ratios were the same (Table I).

Total DNA of parotid glands of rats maintained on L-ISO for 8 days exhibited a different course of change from that of RNA and weight. If rats were only 11–13 days of age at the time of initial injection of L-ISO total DNA of parotid was significantly ($p < 0.01$) lower (30%) after 8 days of ISO than in parotid of untreated litter mates (Table I). After this point in development, however, administration of L-ISO resulted in sharp increases in total DNA of parotid gland, and DNA of the ISO-treated glands was from 1.3 to 1.5 times greater than that of untreated litter mates (Table I). DNA per unit wet weight of tissue (μg/mg) was significantly lower in parotid glands of L-ISO treated rats than in untreated litter mates, regardless of the age at the time of initial injection (Table I).

Administration of the D-isomer in the same dose and for the same periods of time neither altered DNA nor induced statistically significant changes in total RNA or gland size (Table I).

The developmental changes in pattern of DNA and RNA induced by the L-isoproterenol were correlated with mitotic activity and cell size. These determinations were made after only 3 days of ISO when hyperplasia is known to be maximal in the adult gland (11), after 8–9 days, which generally corresponded to the length of time on ISO after

TABLE II. Effect of Chronic Administration of L-Isoproterenol on Mitotic Rate and Cell Size of Parotid During Postnatal Development.[a]

Age at initial injection (days)	Age at sacrifice (days)	No. days on ISO	Mitoses/1000 acinar cells	Cell size (no. nuclei)	Age at initial injection (days)	Age at sacrifice (days)	No. days on ISO	Mitoses/1000 acinar cells	Cell size (no. nuclei)
11	14	3	20 ± 2.1	12 ± 0.7 (5)	2	14–16	12–14	12 ± 1.5	12 ± 0.9 (6)
		0	29 ± 2.3	29 ± 1.0 (5)			0	23 ± 0.9	29 ± 0.3 (9)
14–16	17–19	3	15 ± 1.3	11 ± 0.7 (6)	10–11	19	8–9	15 ± 1.1	11 ± 1.0 (9)
		0	21 ± 1.7	28 ± 1.0 (6)			0	21 ± 1.0	26 ± 0.5 (6)
21	25	3	11 ± 1.1	10 ± 0.5 (5)	14	23	9	14 ± 1.5*	9 ± 1.0 (3)
		0	6 ± 1.4	18 ± 0.7 (5)			0	16 ± 3.0	22 ± 0.9 (6)
29	32	3	6 ± 1.3	10 ± 0.6 (6)	29	34	5	17 ± 0.9	6 ± 0.7 (3)
		0	2 ± 0.7	19 ± 0.3 (6)			0	3 ± 0.5	19 ± 2.0 (3)
					29	36	7	4 ± 1.8*	6 ± 1.0 (3)
							0	2 ± 1.0	19 ± 1.0 (3)
					2	38	36Δ	0*	6 ± 0.5 (3)
							0	1 ± 0	15 ± 0.4 (3)

[a] Values are means ± SE. Number in parentheses refers to number of rats. Saline alone, or L-isoproterenol (ISO) in an average dosage of 13 mg/kg was injected twice daily for the number of days indicated in each case. Comparison was made using the D-isomer in the same dosage for selected intervals; thus, after 8–9 days on D-ISO, mitotic rates were, respectively, 15 ± 4 and 1 ± 0.5, at 23 and 34 days of age (3 rats) and cell number 22 ± 0.9 and 17 ± 1, and no values were significantly different from those of untreated rats ($p > 0.01$). Except in groups indicated with *, L-ISO values were significantly different from values of untreated rats ($p < 0.01$). Cell number is based on number of nuclei per graduated field, and mitotic rate is determined from fields of acini only. Body weight of experimentals did not differ from that of Controls at any age except where indicated by Δ.

which DNA levels were determined, and, additionally at other selected intervals during development. From these data, in Table II, it is clear that administration of L-ISO resulted in a suppression of mitotic activity if rats were less than 15 days of age at the time of initial injection of ISO. Furthermore, this suppression was evident whether ISO was given for as short a period of 3 days or a long as 14 days before sacrifice (Table II).

A suppression of mitotic activity was not observed in rats that were older than 19 days of age at time of initial administration of L-ISO; furthermore, by 25 days of age, mitotic activity of the L-ISO glands was significantly higher than that of controls (Table II).

From the data, in Table II, it is also clear that administration of L-ISO caused cell enlargement in all age groups, and that cell enlargement was evident within 3 days after initial injection of ISO. It is also clear that the increase in cell size effected by 3 days' ISO treatment was generally similar, regardless of the age at which the regimen was instituted. Thus, after 3 days, the number of nuclei per area in parotid of rats 11 or 29 days of age at the beginning of ISO treatment was nearly the same (10–12). If L-ISO administration was continued for longer intervals (5–36 days), the maximal cell size attained depended on the total time of ISO administration as well as the ages at which such administration was initiated and terminated. A maximal increase in cell size (5–6 nuclei per area) was only attained if the ISO regimen was initiated after weaning, or, if initiated at a very early age (2 days), continued well beyond weaning.

In order to determine the normal role of β-adrenergic receptors in postnatal parotid development, the β blocking agent, propranolol, was chronically administered for 12–20 days to rats 3–5 days of age at time of initial injection. From the data in Table III it is clear that neither gland size, total DNA nor RNA was modified by this regimen, and values of the propranolol-treated glands did not differ from those of untreated litter mates.

Discussion. The data show that chronic administration of L-isoproterenol during postnatal development alters mitotic rate and consequently total DNA of rat parotid. The specific nature of the alteration, however, is related to the phase of postnatal growth during which L-isoproterenol administration is initiated. If mitotic rate is high and cells are in the process of differentiation, as they are during the growth phase between birth and weaning (4, 5), administration of large doses of the L-isomer of isoproterenol causes an inhibition in mitotic rate and decrease in total DNA of the gland; gland and cell size, total RNA and micrograms RNA per milligram wet weight are, however, increased. When initiation of the L-ISO regimen is delayed until after weaning, when proliferative activity has dropped considerably (5), and the gland bears a close resemblance to that of adult, the response to L-ISO during this

TABLE III. Effects of Chronic Administration of Propranolol on Parotid Weight, Total DNA and RNA During Postnatal Development.[a]

Age at initial injection (days)	Age at sacrifice (days)	No. days on propranolol	Total parotid		
			Wet wt (mg)	DNA (μg/gland)	RNA (μg/gland)
3	16	13	18 ± 1	141 ± 8	220 ± 5 (5)
		0	20 ± 1	158 ± 7	232 ± 28 (8)
5	17	12	22 ± 1	208 ± 14	245 ± 18 (4)
		0	22 ± 1	175 ± 6	223 ± 15 (4)
3	23	20	62 ± 2	394 ± 12	540 ± 20 (4)
		0	64 ± 2	411 ± 10	530 ± 35 (4)

[a] Values are means ± SE. Number in parentheses refers to number of rats. In no case did the experimentals differ from controls ($p > 0.01$).

period resembles that of the adult: cells enlarge to the same maximum, total RNA and DNA of the gland increase, and mitotic activity is markedly enhanced.

The acceleration of differentiation by ISO may be related to the suppression of cell division induced by this agent, and in fact may be the consequence of this suppression. It is nonetheless difficult to account for the suppression of mitosis by ISO, an agent that normally is a very potent stimulator of DNA synthesis and mitotic activity (12, 13). A possible interpretation of these results may be made by examining Radley and Hodgson's (14, 15) recent findings concerning effects of racemic isoproterenol on adult submaxillary. They found that cells of the adult gland did not undergo more than one or two divisions when treated with isoproterenol (14), and furthermore, when the ISO-stimulated cells were in G_2 at the time of a second injection of isoproterenol, movement of the cells through G_2 into mitosis was blocked (15). In addition, the interval of block could be extended with repeated doses of isoproterenol (15). Thus, if these conclusions can be applied to the present data, it is possible that if cells are stimulated with isoproterenol when they are dividing as part of a normal growth pattern (as in the early postnatal parotid) they do not complete their division and suppression of mitosis is observed. With repeated injections, a decrease in total DNA thus can ultimately occur.

Not all normally dividing systems would be expected to exhibit this response to isoproterenol and, in fact, Radley and Hodgson (14) found no effect on the crypt cells of mouse duodenum. The presence of β-adrenergic receptors, on the other hand, may be a prerequisite for isoproterenol-induced suppression of normal cell division. In fact, the spatial orientation of these sites appears to be critical for this action of isoproterenol as well as its action in inducing mitosis since D-ISO is essentially without effect. β-adrenergic receptors do not, however, appear to have a critical role in normal postnatal development of parotid, since administration of large doses of the β antagonist, propranolol, during postnatal development produced no changes in gland size or cell number. This is not surprising since the sympathetic innervation itself does not have an important role in postnatal changes in cell number and gland size (16). The administration of the large doses of isoproterenol may in fact cause a premature response at β adrenergic receptor sites. According to observations of Iversen *et al.* (17), a dense but immature sympathetic innervation appears to be present in salivary glands at a very early age.

Finally, it is also clear from the present data on parotid gland that levels of total RNA, RNA per milligram of tissue, and size of cells and whole gland can increase even under conditions when total DNA is decreased and mitosis is inhibited. This is the situation when L-isoproterenol is chronically administered during a postnatal period before weaning. Thus, with the use of isoproterenol, the parotid gland, in this early phase of postnatal development, may provide a unique system for study of the processes of hypertrophy and hyperplasia.

Summary. Administration of L-isoproterenol for 8 days during the early phase of postnatal development when mitosis is normally high and cells are in the process of differentiation leads to suppression of mitotic rate and decrease in gland levels of DNA. Acceleration of differentiation, with increases in total RNA, cell and gland size were also observed. The accelerated differentiation may be the consequence of the inhibition of mitosis. If administration of isoproterenol is delayed until after weaning when cell division is markedly decreased and cells more nearly resemble those of adults, the usually expected hyperplasia as well as hypertrophy are observed. The spatial orientation of the β-adrenergic receptors appears to be a critical factor in these growth responses, since the D-isomer, injected in the same doses as the L-isomer, failed to cause significant effects. Finally, chronic administration of propranolol does not modify the course of postnatal growth in rat parotid, suggesting that β adrenergic receptors normally have little role in postnatal development of parotid.

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