β-Adrenergic Effects by Autonomic Agents on Mitosis and Hypertrophy in Rat Parotid* (33806)

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Chronic administration of large doses of the sympathetic agent, isoproterenol, produces marked enlargement of the salivary glands of rat (1) and mouse (2). Recently, evidence has been provided which indicates that β-adrenergic agents generally cause salivary gland enlargement while catecholamines that act primarily at α-adrenergic receptors do not (3). Gland enlargement by catecholamines has therefore been attributed to an action mediated through β-adrenergic receptors.

However, gland enlargement can also be induced by other autonomic agents. Thus, pilocarpine, a parasympathomimetic agent, can induce an increase, albeit small, in gland size (4). Pilocarpine, however, has in addition to parasympathomimetic properties, β-adrenergic effects that are mediated via the superior cervical ganglion (5–7). However, since pilocarpine is more effective than the catecholamines in promoting secretory activity, it is possible that the mechanism involved in the catecholamine-induced enlargement differs from that involved in the pilocarpine-induced enlargement and work hypertrophy, rather than β activity, might account for the increased gland size (8, 9). The present work was undertaken to determine whether the mechanism involved in both cases was the same.

In addition, since it has been shown that, with the β-adrenergic agent isoproterenol at least, hyperplasia and hypertrophy both contribute to the gland enlargement (1, 3, 9–13), the role of hyperplasia and hypertrophy in enlargement effected by parasympathomimetic and sympathomimetic agents was investigated.

Materials and Methods. Long-Evans female rats, 6 months of age, and averaging 210 ± 3 (SE) g were used in these experiments. They were maintained on a diet of lab chow and water *ad libitum*. Saline injected or untreated controls were used. The sympathomimetic agents, epinephrine, phenylephrine, and isoproterenol (ISO) were administered ip, in volumes of 0.3–0.5 ml, 3 times daily in the following doses (mg/injection): epinephrine, 0.06; phenylephrine, 0.5 and 2; isoproterenol, 0.08, 0.5, 3, and 4. With the parasympathomimetic agents, pilocarpine nitrate (PC) and bethanechol (Urecholine) (UR) doses (mg/injection), were 3.3 and 10 for pilocarpine and 0.33 for Urecholine. The adrenergic blocking agents were administered 20 min prior to injection of the agonists in the following doses (mg/injection): phenoxybenzamine hydrochloride (Dibenzyline) (DI) 2; propranolol (Inderal) (IN) from 2.5, 4, 5.5, depending on the dose of the agonist used. The agents were administered for a period of 3 or 10 days; an 18-hr drug-free period preceded sacrifice of the animals. Animals were anesthetized with 1% Nembutal and parotid glands were rapidly removed and weighed on a torsion balance. Part of the tissue was preserved in Bouin's for histological examination; tissues were cut 6 μ in thickness and stained with hematoxylin and eosin. Mitotic counts were made using a calibrated eyepiece micrometer, and sections containing only acinar cells were counted. Nuclear size was determined with the Filar micrometer. Cell size was estimated in 2 ways: by direct measurement of height and width of acinar cells, and by counting the number of nuclei per calibrated area. This second method was particularly satisfactory under conditions where nuclear size remained constant. However, in experiments where isoproterenol was used, nuclear size did not remain constant but increased at doses above 80 μg. Nuclear size was related to size of dose as well as length of the period during

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TABLE I. Changes in Mitotic Index, Acinar Cell Size, and Gland Size of Rat Parotid Following Chronic Administration of Autonomic Agents, and Antagonists.[a]

Agent	Daily amt (mg)[b]	Days on drug	Parotid wt. (mg)	Mitotic[c] index	No. of nuclei/field[d]	Cell size (μ)		Nuclear size (μ)	No. of rats
						Height	Width		
None			208 ± 5	0.3 ± 0.1	14 ± 0.3	14 ± 0.4	14 ± 0.4	5.8 ± 0.06	8
Sympathomimetic									
ISO	0.24	3	247 ± 5	3 ± 0.1	13 ± 0.5	15 ± 1	16 ± 1	6.1 ± 0.07	4
	0.24	10	284 ± 27	0	7 ± 0.2	20 ± 0.1	21 ± 1.3	6.1 ± 0.12	4
	1.5	3	361 ± 22	56 ± 1	7 ± 0.2	18 ± 0.6	19 ± 0.1	7.3 ± 0.15	6
	1.5	10	530 ± 35	0	4 ± 0.2	22 ± 1.5	22 ± 0.7	7.8 ± 0.11	5
	7.0	3	640 ± 20	76 ± 3	6 ± 0.2	17 ± 0.1	19 ± 1	8.7 ± 0.15	3
	12.0	3	488 ± 32	78 ± 2	6 ± 0.2	17 ± 0.8	19 ± 1	8.8 ± 0.15	9
	12.0	10	1186 ± 94	0	3 ± 0.1	25 ± 0.1	28 ± 0.1	11.1 ± 0.20	10
Epinephrine	.18	3	175 ± 7	0	15 ± 0.5	14 ± 0.9	13 ± 0.6	5.6 ± 0.06	4
	.18	10	174 ± 7	0.3 ± 0.1	15 ± 0.4	14 ± 0.6	12 ± 0.5	5.6 ± 0.06	4
Phenylephrine	1.5	3	163 ± 5	0	16 ± 0.7	14 ± 0.5	14 ± 0.8	6.3 ± 0.18	3
	1.5	10	172 ± 5	0	15 ± 0.6	13 ± 0.8	14 ± 0.8	6.2 ± 0.01	3
	6.0	3	112 ± 5	0	20 ± 0.8	13 ± 0.9	14 ± 0.8	6.4 ± 0.16	4
Parasympathomimetic									
PC	10.0	3	234 ± 4	1.5 ± 0.2	11 ± 0.2	15 ± 0.6	16 ± 0.7	5.8 ± 0.07	7
	10.0	10	265 ± 18	0	9 ± 0.3	18 ± 0.9	17 ± 0.8	5.8 ± 0.06	4
	30.0	3	258 ± 5	0	14 ± 0.3	15 ± 0.4	15 ± 0.5	5.7 ± 0.08	4
UR	1.0	3	241 ± 6	0.8 ± 0	12 ± 0.4	14 ± 0.5	16 ± 0.6	5.7 ± 0.06	7
	1.0	10	199 ± 9	0	15 ± 0.4	14 ± 0.5	14 ± 0.5	5.5 ± 0.06	4
Antagonists + ISO or PC									
IN, PC	12, 10	3	140 ± 2	0	20 ± 0.5	13 ± 0.2	14 ± 1	5.6 ± 0.15	3
IN, DI, ISO	16, 6, 7	3	241 ± 16	0	10 ± 0.4	16 ± 0.9	16 ± 1	6.0 ± 0.12	3
IN, ISO	16, 7	3	238 ± 19	0.3 ± 0.1	9 ± 0.2	17 ± 0.1	17 ± 2	6.3 ± 0.16	4
IN, ISO	12, 7	3	418 ± 28	0.5 ± 0.1	9 ± 0.5	17 ± 0.7	15 ± 0.9	5.5 ± 0.08	5
IN, ISO	7, 12	3	386 ± 10	37 ± 2	5 ± 0.2	17 ± 0.3	14 ± 0.9	7.3 ± 0.03	3

[a] All values are means ± SE. Abbrev.: isoproterenol, ISO; pilocarpine, PC; Dibenzyline, DI; Inderal, IN; Urecholine, UR.

[b] Total administered ip in 3 divided doses daily. When more than one agent was administered, order given represents order of injection; injection of antagonists were given ip 20 min prior to injection of autonomic agent.

[c] Number of mitoses per 1000 acinar cells; counts were made of 10 areas from 2 slides from each rat; 10 cells or nuclei of 2 slides from each rat were also measured.

[d] Number of nuclei in calibrated field of acinar cells only.

which the drug was administered. After 3 days of administration of isoproterenol in doses ranging from 0.5 mg to 6 mg /injection, nuclear size had increased to about 7.5 μ, and by 10 days, had reached a size of 10–11 μ with the highest dose (Table I).

Results. As previously shown (10), isoproterenol (ISO) caused an increase in gland size that was significant after 3 days of drug administration; the magnitude of the increase was generally dependent on the concentration used (Table I). Thus, with a total daily dose of 0.24 mg (80 μg/injection) an increase (over controls) of only 19% was observed; with 1.5 mg/day (500 μg/injection) there was a 74% increase (over controls); at the highest concentration, gland weight was increased 135%. By 10 days, increases were more marked, ranging from 40 to 500%, depending on dose.

Increases in cell number and cell size accompanied the gland enlargement. The increase in cell number with isoproterenol has previously been shown to be most conspicuous during the first 2–4 days after initiation of treatment (10–13). Thereafter, hyperplasia decreased in importance and the concurrently occurring hypertrophy became the dominant, in fact, nearly exclusive event by 8–10 days. Mitotic index, after 3 days of ISO, was conspicuous (3 $\pm$ 0.2/1000 acinar cells) even at the lowest dose (80 μg), and increased to 56 and 78/1000 at the higher doses. Cell size increased concurrently during this early period, but the increase was not significant at doses lower than 500 μg/injection (Table I). After 10 days of ISO regimen, evidence of mitotic activity was virtually absent at all concentrations of ISO used, but cell size, on the other hand, was conspicuously increased at all concentrations. With high doses, cell size showed a 50–85% increase from control levels, and even with doses of only 80 μg (240 μg/day), cell size was significantly increased ($p < .01$). This was evident from measurements of height and width as well as number of nuclei/area (Table I).

It is clear from the data in Table I that prior administration of the β-adrenergic blocking agent, Inderal, could completely suppress the mitosis induced by ISO, provided the dose of Inderal was adequate. Thus, 16 or 12 mg (per day) of Inderal completely suppressed the effects of 7 mg (per day) of ISO; when the ratio was changed (7 mg of Inderal to 12 mg of ISO), mitotic activity was only partially suppressed (about 50%). (On an equal weight basis, 1 mole of ISO = 0.85 moles Inderal). The hypertrophic effect on the other hand was not completely suppressed at any dose relationship used (Table I). It is also of interest that the secretory response to 7 mg of ISO (daily) could be prevented by prior administration of only 7 mg of Inderal. Thus, at dose levels of Inderal sufficiently high to block mitosis induced by ISO, or the secretory response, complete inhibition of the hypertrophic effect on the acinar cells was not observed (Table I). This was clear from both parameters (height, width as well as number of nuclei) measured. It may be noted that only where mitotic activity still remained (with Inderal) was there appearance of increased nuclear size. The effects on cell size and number induced by ISO were no more altered by dibenzyline + Inderal than by Inderal alone (Table I). In marked contrast to isoproterenol, epinephrine and phenylephrine, which act principally on α-adrenergic receptors, caused a decrease in gland weight which, after 3 or 10 days of low doses of the drugs amounted to 15%. With the very high dose (6 mg) of phenylephrine, the decrease in gland weight became very marked (50%). These agents also caused no change in mitotic activity from that observed in controls, and only slight decreases in cell size (Table I). Again, only with the very high dose of phenylephrine were the decreases marked.

Although gland size, acinar cell size and mitotic activity were increased by chronic administration of the parasympathomimetic agents, pilocarpine and Urecholine, the changes were small when compared with those effected by isoproterenol. After 3 days of administration of Urecholine or pilocarpine, the parotid glands were about 15% larger than those of control rats; after 10 days, the glands of pilocarpine-treated rats were even

larger (22% greater than controls) but those of rats treated with Urecholine were not different from those of controls (Table I). Mitotic activity in these glands was only slightly greater than that of control glands after 3 days of drug administration; after prolonged treatment (10 days), values were indistinguishable from those of controls (Table I). It may be pointed out that both doses of pilocarpine used were supramaximal and caused copious salivary secretion (flow rates of saliva exceeding 0.06 mg/min/mg of gland). After 3 days of Urecholine administration, only slight but statistically significant ($p < .01$) increases in cell size were observed; and by 10 days, however, no change was at all evident. With pilocarpine, changes in cell size were more marked. A significant decrease in number of nuclei per area provided evidence of the increased acinar cell size at 3 and 10 days of pilocarpine treatment. The data based on height and width measurements were, at 3 days, also suggestive of increased cell size; these became statistically significant differences by 10 days ($p < .01$). Prior administration of the β-adrenergic blocking agent, Inderal, completely prevented the small mitotic and hypertrophic effects of pilocarpine.

Discussion. The data show that autonomic agents which cause salivary gland enlargement apparently do this through the mediation of β-adrenergic receptors. This is true not only for adrenergic agents (3), but for agents usually considered to be primarily parasympathomimetic but which can act also through the sympathetic ganglion (5–7). Thus the enlargement induced by isoproterenol which acts directly on gland β-adrenergic receptors, as well as that induced by pilocarpine which acts indirectly, can be prevented by prior administration of the β-blocking agent, Inderal. On the other hand, agents that act primarily on α-adrenergic receptors, cause a decrease or no change in gland size. Such effects of α-adrenergic agents had previously been reported for submaxillary (3, 14); only when an α-blocking agent was administered prior to the agonist was an increase in gland size seen (3).

The gland enlargement which is produced in response either to the adrenergic or the parasympathomimetic agents does not appear to be necessarily related to the degree of secretory activity induced in the gland, although such activity may play some role under other circumstances (4, 11, 15–19). In the present work, the use of a β-adrenergic blocking agent, administered before pilocarpine, suppressed enlargement of the gland without appreciably diminishing secretory rate. Furthermore, isoproterenol caused gland enlargement at dose levels that are too low to produce visible secretory activity, and which are far less than those used with pilocarpine or α-adrenergic agents. In addition, the increases in gland size seen under such conditions are greater than those observed with high doses of pilocarpine.

It is well known that the gland enlargement caused by isoproterenol involves an increase in size and number of acinar cells (1–3, 8–13); hyperplasia, dominant initially, is accompanied and superseded by hypertrophy (11–13). From the present work it is clear that the increased gland size caused by the parasympathomimetic agents also involves hyperplasia and hypertrophy, and in the same sequence; only the magnitude is less. Furthermore, with both groups of drugs, the mitotic and hypertrophic effects are mediated through β-adrenergic receptors. Thus the mitosis and hypertrophy usually induced by pilocarpine or isoproterenol can be completely suppressed with the β-adrenergic blocking agent, Inderal. Further, the data show that the mitosis can be blocked more readily than the hypertrophic effect, since at doses of IN that effectively inhibit mitosis, cell size continued to increase. Finally, mitotic and hypertrophic effects are not related to degree of secretory activity since, on the one hand, both can be seen when secretory activity is absent (as with low doses of ISO); on the other hand, when secretory activity is excessive (as with high doses of pilocarpine), the mitotic and hypertrophic effects are minimal. The intensity of the mitotic response seems to be related to the number of β receptors activated.

Summary. A series of sympathomimetic and parasympathomimetic agents were administered chronically to rats. At 3 and 10 days, the effects of these agents [the β-adrenergic drug, ISO; the primarily α-adrenergic drugs, epinephrine and phenylephrine, and the parasympathomimetic agents, pilocarpine (PC) and Urecholine (UR)] on parotid gland weight, and size and mitotic activity of acinar cells were examined. It was found that mitotic activity was observed only under those conditions where activity of β-adrenergic receptors could be implicated (ISO, PC, and UR), and the β-blocking agent, Inderal, was used to demonstrate this point. Increases in cell and gland size more generally accompanied β-adrenergic activity and were evident even in the absence of a distinct mitotic response; α-mediated effects, however, produced no mitotic effects but instead decreased cell and gland size. Secretory activity was not necessarily involved in any of the β-adrenergically mediated increases in gland size, acinar cell size or number.

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