Salivary gland changes after isoproterenol-induced enlargement

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Salivary glands and cardiac ventricles were examined after chronic administration of pharmacological doses of isoproterenol (ISO) to young adult female rats. Enlargement of glands was observed, generally confirming previous reports. Enlargement of ventricles also occurred. Salivary glands were differentially affected, with parotid showing the most, and sublingual the least enlargement. Histological examination revealed increase in cell size sufficient to account for the increase in organ weight, although some mitotic activity was noted. Determination of water, electrolyte, and amylase content of glands indicated the hypertrophy is not due simply to water imbibition; changes in gland composition, when seen, were generally reminiscent, in magnitude and direction, of those occurring ordinarily during function activity. Function after chronic treatment with ISO was little affected; reduction in flow rate after pilocarpine was the major change observed in enlarged glands. Changes in gland and ventricle size and gland composition and function, induced by chronic ISO treatment, were largely reversible by withdrawal of the drug. It appears that in the adult, isoproterenol effects increase in organ size mainly by increase in cell size.

Salivary gland enlargement, induced by chronic administration of the adrenergic agent, isoproterenol, has been reported recently in rat (1) and mouse (2). Chronic stimulation involving the sympathetic innervation of rat submaxillary gland has also been reported (3) to cause enlargement. With regard to isoproterenol-induced enlargement, Selye, Veilleux, and Cantin (1) have suggested that, at least in the rat, this is selective for salivary glands and results primarily from increased cellular proliferation. In a later report on the mouse, Brown-Grant (2) indicated that hypertrophy alone may account for the increased gland size. Determination of the mechanism of this enlargement is pertinent to evaluation of the role of the sympathetic nervous system in regulation of normal growth. Accordingly, detailed investigation of the effects of isoproterenol on rat salivary glands, and to a lesser extent on heart ventricle, has been undertaken to elucidate the nature and specificity of the organ enlarging action. Composition and functional status, as well as histological appearance, particularly of the salivary glands, were examined.

METHODS

Long-Evans rats, generally females, 4-6 months of age, were kept as controls or placed on a regimen of twice-daily intraperitoneal injection of an aqueous solution of isoproterenol hydrochloride (3,4-dihydroxyphenyl-ethanol-isopropylamine) for periods ranging from 6 to 14 days. Daily dose (per 200-230-g animal) ranged from 6 to 12 mg. After 6-14 days of isoproterenol treatment, use of the drug was discontinued (usually for 36-48 hr) in preparation for assay of composition or secretory capacity of the glands. These animals and untreated controls were fasted for the last 24 hr prior to acute experimentation and were then lightly anesthetized with Nembutal. For both parotid and submaxillary, one whole gland of each pair was removed and weighed on a Roller-Smith torsion balance, and fragments were appropriately preserved for histological examination and analysis of amylase, electrolytes, and water content.

The remaining gland of one or both pairs was prepared for collection of the secretion. For this the duct was freed and saliva was collected by micropipette (4), or tared bottle (5), after intraperitoneal administration of pilocarpine nitrate (2.2-2.4 mg/rat), or isoproterenol (2-25 mg/rat, with doses of 8-10 mg/animal usually employed). Flow rate is expressed as microliters per milligram of gland per minute (μl/mg min). Samples of salivary secretion were diluted in phosphate-buffered saline for determination of amylase (6), or in ion-free water for electrolyte analysis by flame photometry (7). Gland tissue samples, taken before or after acute stimulation, were homogenized (6) for estimation of amylase, or dried at 105°C for analysis of water and subsequently dry-ashed (7) at 525°C for analysis of electrolytes. Fragments preserved for histological section were fixed in Zenker's or Bouin's fluid and stained with iron hematoxylin and phloxine or hematoxylin and eosin.
Hearts were removed from controls and from animals of similar age and sex that had been stimulated chronically with isoproterenol. The cardiac ventricles were separated from the atria and blood vessels and blotted before weighing.

RESULTS

Tissue changes after chronic isoproterenol treatment. Marked enlargement of rat salivary glands resulted from chronic administration of isoproterenol (ISO), generally confirming observations reported by Selye et al. (1). However, the doses used to cause this enlargement (6-12 mg/animal/day over a period of 6-14 days), although very much greater than doses employed to investigate cardiovascular dynamics (8, 9), were markedly lower than those reported by Selye et al. In fact, daily doses in excess of 12-16 mg/animal caused a high death rate, especially among males; hence, for investigation of chronic effects of ISO, only females were used. Following this ISO treatment, enlargement of all three salivary glands was observed. In apparent contradiction to the previous report (1), however, the degree of enlargement varied, with parotid exhibiting the greatest size increase [usually 3- to 5-fold, as previously reported (1)]; submaxillary an intermediate increase (2- to 3-fold); and sublingual a small increase, as compared with glands of control female animals. Higher dose and longer duration of treatment, within the range indicated, effected more marked increases (Table 1). Previous work (9a) has shown some heart enlargement after administration of isoamyl alcohol for only 2 days.

Histological examination of submaxillary (Fig. 1) and parotid glands at intervals during the period of chronic ISO administration revealed progressive increase in cell size, particularly of acini, to an extent which would appear to account for the observed increase in gland weight (2). Fibers of enlarged ventricles also were greater in diameter than those of normal. In glands, striated duct cells showed no obvious increase in size; ducts generally became increasingly compressed. Granular tubules of submaxillary became indistinguishable from acini after 10-12 days of treatment (Fig. 1B). In later stages of gland enlargement, in submaxillary only, areas of necrosis were sometimes observed. Mitotic figures were visible in submaxillary and parotid gland in early stages of ISO treatment, but were not usually evident in later stages of enlargement (Fig. 1B). It was noted that, in stages showing mitotic figures, cytoplasmic furrowing did not appear to accompany telophase.

In view of the apparent paucity of cytoplasmic divisions, effects of discontinuation of ISO treatment were investigated. Marked reversal of enlargement by 10-17 days after drug withdrawal was observed grossly (Table 1) and histologically (Fig. 1C) in salivary glands ("reversed" glands) and in ventricle. In the glands, return toward normal differentiation as well as cell size occurred (Fig. 1C). In addition, a few cell counts in sections of normal and reversed glands showed similar numbers of acinar cells in the two. Apparently hyper trophy, possibly associated with some degree of polyploidy (10) or even true hyperplasia (1), is the major change involved in the size increase in the glands.

Characteristics of the increase in cell size were further investigated by analyses of water, sodium, potassium, and amylase in the salivary glands. In enlarged glands these factors were constant over a period of from 12-60 hr after the last ISO injection. For convenience, 36-48 hr were allowed to intervene between the last ISO injection and determination of composition or further experimentation on the enlarged gland.

Water content, in relation to dry weight, showed no increase in enlarged glands as compared with normal controls (Table 2), thus eliminating water imbibition as the sole cause of the enlargement. In enlarged submaxillary gland, water content was in fact decreased, to a degree reminiscent of that observed in normal gland during secretory activity following acute pilocarpine stimulation (7). Reversed glands in both cases showed a water content, in the absence of acute stimulation, again equal to that of normal controls (Table 2).

Although water content in relation to dry weight did not increase during enlargement, total water was of course greater in the larger, ISO-treated glands, and total gland dry weight, probably representing mainly protein, then also was increased. Total gland amylase, however, did not increase concurrently with total dry
weight. Amylase concentration in parotid gland after enlargement was reduced appreciably (Table 2) even when compared with levels in normal glands after acute pilocarpine stimulation (6). This may indicate continuing influence of the stimulating agent even though secretion is not obvious. Reversal of the size increase restored parotid amylase levels to normal resting values (Table 2).

Sodium and potassium levels of enlarged glands were somewhat reduced from normal, as shown in Table 2. The lowered sodium can be correlated, at least in part, with histologically evident reduction in extracellular space, especially marked in parotid. The potassium levels in enlarged submaxillary and parotid glands are suggestive of those usually observed in normal glands after acute pilocarpine stimulation (7). In any event sodium and potassium levels of the enlarged glands did not show changes corresponding to the changes in gland size, and the electrolytes therefore seemed to be accumulated, in the whole gland during enlargement, approximately to the same extent as tissue total solids. Reversed glands showed a return to control levels of Na and K (Table 2).

Changes in secretory activity of salivary glands after chronic isoproterenol treatment. Flow of saliva was observed during the period of chronic isoproterenol administration, in accord with the observation of Selye, Veilleux, and Cantin, who described the flow as “copious” (1). Termination of ISO treatment was followed within 3-4 hr by cessation of saliva flow. After this, flow could be initiated again by restimulation, either by pilocarpine or ISO. Secretory capacity of enlarged glands was investigated 36-48 hr after the end of chronic ISO treatment. At this time, flow rate of saliva in response to a single injection of pilocarpine was appreciably lower from enlarged than from normal glands, when these were compared on an equal weight basis. This difference was particularly marked early in the period of salivary flow. However, comparison of volumes of fluid collected, without correction to the same weight basis, showed that these could be similar for enlarged and normal glands. Data for submaxillary and parotid flow rates are given in Table 3.

Stimulation by a single injection of ISO (8-10-mg doses usually employed, but doses could vary from 2 to 25 mg, with little alteration in flow rate) caused secretion by enlarged and normal salivary glands. Normal glands showed a lower flow rate after acute ISO stimulation than after pilocarpine, but enlarged glands seemed equally affected by both agents (Table 3). Salivary flow in both normal and enlarged glands could be sustained for several hours following a single injection of ISO.

Examination of sodium and potassium levels in submaxillary and parotid salivary fluids, obtained by acute stimulation with pilocarpine or ISO, showed only small differences from normal in the secretion of these electrolytes by enlarged glands (Table 3). A striking effect of ISO stimulation on concentration of potassium in submaxillary and parotid secretions from enlarged and...
These elevated potassium levels appeared with widely proliferative Brown-Grant enlargement to be primarily the result of cellular processes.

**DISCUSSION**

The results obtained here confirm the observation that chronic administration of pharmacological doses of isoproterenol causes enlargement of salivary glands (1, 2) and elucidate the nature and organ specificity of this effect. With regard to the nature of isoproterenol action, Selye, Veilleux, and Cantin (1) reported a marked increase in mitotic figures, as well as in cell size, in enlarged salivary glands, but considered the enlargement to be primarily the result of cellular proliferation. Brown-Grant (2), however, found no clear evidence of increased mitosis in isoproterenol-enlarged mouse salivary glands. The present experiments show the presence of mitotic figures early in the period of isoproterenol treatment but fail to support the occurrence of appreciable cell proliferation. An increase in cell size sufficient to account for the increase in gland size was observed here. This work then supports Brown-Grant's contention that gland enlargement is primarily the result of the increase in size of individual cells, probably related to the increased functional activity. The essential reversibility of the increase in size, for the whole gland and individual cells, as well as the reversibility of the biochemical and functional changes noted, are consistent with the view that isoproterenol-induced enlargement is due mainly to increased size of individual cells.

That the hypertrophy is not simply the result of water imbibition is indicated by the nature of the changes in gland composition observed after cessation of chronic isoproterenol treatment and associated secretory flow.

**TABLE 2. Electrolyte, water, and amylase content of salivary glands after chronic isoproterenol**

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Normal (12)</th>
<th>Enlarged (12)</th>
<th>Reversed (5)</th>
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<tbody>
<tr>
<td>Na (mEq/l)</td>
<td>35.5 ± 0.9</td>
<td>24.1 ± 0.9</td>
<td>37.9 ± 1.3</td>
</tr>
<tr>
<td>K (mEq/l)</td>
<td>91.0 ± 0.9</td>
<td>71.2 ± 1.5</td>
<td>89.3 ± 1.8</td>
</tr>
<tr>
<td>H₂O/Dry</td>
<td>3.3 ± 0.0</td>
<td>2.6 ± 0.05</td>
<td>3.3 ± 0.06</td>
</tr>
</tbody>
</table>

Values are given as means ± se and are mEq/kg fresh tissue for Na and K, and mg reducing sugar (as glucose) formed in 15 min per mg fresh tissue, for amylase. Analyses were made on glands referred to in Table 1 and categories are defined there; enlarged glands are from group on ISO for 10-14 days. Figures in parentheses indicate number of rats used. *5 rats used.

normal glands was noted, however. In ISO-stimulated parotid secretion, potassium rose to values 50-100% greater than those of pilocarpine-stimulated saliva, whereas in submaxillary secretion the increase was even greater, with potassium levels generally approaching concentrations in intracellular water (11) (Table 3). These elevated potassium levels appeared with widely varying doses of drug (2-25 mg/animal), and, moreover, cannot be solely attributed to influence of flow rate (Table 3). A similarly marked increase was noted in amylase level of parotid saliva, obtained after acute ISO stimulation. It is possible that ductal transport, especially of water (I), is affected by isoproterenol treatment and associated secretory flow.

Some differences were noted in electrolyte levels of salivary fluids and gland tissue of the normal young adult female rats when compared with those of older animals, generally males, used for previous investigations (5, 7). Age, and possibly sex, may then be important factors in regulating these levels. Parotid gland seemed particularly affected. In young adult females, saliva from this gland characteristically showed lower Na and higher K levels (footnote, Table 3). Parotid gland levels of Na also tended to be lower in the young female than in older (male) animals, and short-term pilocarpine stimulation then effected an increase rather than a decrease (7) in tissue sodium.

**TABLE 3. Flow rate and composition of saliva from acutely stimulated normal and enlarged glands**

<table>
<thead>
<tr>
<th>Flow Rate, μl/min</th>
<th>Normal Stimulation</th>
<th>Isoproterenol Stimulation</th>
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<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Na, mEq/l</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>K, mEq/l</td>
<td>63</td>
<td>72</td>
</tr>
<tr>
<td>Flow Rate, μl/min</td>
<td>±.007</td>
<td>.009</td>
</tr>
<tr>
<td>Parotid saliva</td>
<td>102</td>
<td>62</td>
</tr>
<tr>
<td>Amylase*</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Initial and final – secretion collected within 5 min, or at least after 15 min, respectively, following injection of stimulating agent. Values are given as means ± se; figures in parentheses give number of rats. *Amylase activity expressed as mg of reducing sugar, as glucose, formed in 15 min per ml of fresh saliva. Parotid saliva, analyzed for electrolytes, are from 4-6-month-old females. In 22 males, electrolyte levels in parotid saliva, obtained by acute pilocarpine stimulation, were different, with mean levels for Na, 132 mEq/liter (range, 110-150); K, 19 mEq/liter (range, 11-25). Sex or age difference did not appreciably modify electrolyte levels of saliva from submaxillary glands.
Changes were generally relatively small and are consistent with those observed normally after acute parasympathomimetic stimulation, suggesting continued manifestation of the stimulatory effects initiated during the period of chronic isoproterenol administration. Thus the biochemical status of the hypertrophied gland is similar to that of the active normal gland.

Function seems little changed from normal in enlarged glands. After acute pilocarpine stimulation, salivary flow rate is reduced. This decrease may be related to failure of chronic isoproterenol to induce noticeable hypertrophy of striated ducts, since these ductal elements have been strongly implicated in fluid and electrolyte secretion (12, 13). The similarity of volume flow (flow rate uncorrected for inequalities in gland weight) from normal and enlarged glands is consistent with this view.

With regard to organ specificity of the enlarging effect of isoproterenol, it does not appear that this effect is limited to salivary glands, in view of the hypertrophy of cardiac ventricle observed also. It seems likely that enlargement of both salivary glands and ventricles is related to increased functional activity. In salivary glands copious and prolonged secretion is observed in response to administration of physiological doses at least, rate and amplitude of cardiac contraction are increased (8, 9, 14). Whether the enlargement is due to hyperfunction in general, or specifically to hyperfunction induced by sympathomimetic stimulation, cannot be decided on the basis of these data. Elucidation of a possible normal role of sympathomimetic agents in establishment of organ size must await further work.

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REFERENCES