THE INFLUENCE OF ISOPRENALEINE AND PROPRANOLOL ON THE SUBMAXILLARY GLAND OF THE RAT

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It has been pointed that adrenergic β-receptor is greatly concerned in the salivary secretion of amylase, which is one of the main digestive enzymes (1).

It has also been noticed that the repeated administrations of isoprenaline which has a strong affinity to β-receptor for catecholamines show a marked enlargement of the salivary glands (2~11). On the other hand, it has been also demonstrated that dibenamine, one of the potent α-adrenergic blocking agents, and guanethidine, an antiadrenergic agent, show a gradual atrophy in the salivary gland after the chronic administration (11, 12).

Although there are many reports concerning the enlargement of the submaxillary glands treated with isoprenaline, little has been known whether this enlargement is due to the β-action of isoprenaline and whether it produces an enzymatic alterations in the enlarged salivary glands.

The present study deals with the effects of β-adrenergic blocking agent on the development of the enlargement induced by the chronic administration of isoprenaline in rat submaxillary glands from the histological, histochemical, biochemical and pharmacological points of view.

MATERIALS AND METHODS

The experimental animals were 250 female Sprague-Dawley albino rats weighting about 150 g. The experiments consisted of the following six groups were performed.

Group I: A dose of 5 mg/kg of isoprenaline (ISO) was administrated once daily for 14 days.

Group II: A dose of 30 mg/kg of propranolol was administrated once daily for 14 days.

Group III: Doses of 5 mg/kg of ISO and 30 mg/kg of propranolol were administrated once daily for 14 days.

Group IV: Doses of 5 mg/kg of ISO and 20 mg/kg of nialamide were administrated once daily for 14 days.

Group V: Distilled water was injected once daily as control for 14 days.

The observations were made on the 7th day and 14th day respectively in the above five groups.
Group VI: A dose of 80 mg/kg of ISO was administrated once daily for 7 days.

At the end of the experiment, the rats were starved for the last 24 experimental hours and were slaughtered to death on the head. Bilateral submaxillary glands including the sublingual glands were immediately removed. The submaxillary gland was weighed in a torsion balance after the sublingual gland was separated.

For the histological observation, the specimens were fixed with Bouin solution. The fixed tissues were embedded in paraffin and were sectioned at 4 μ. In both histochemical and biochemical studies, the removed submaxillary glands were rapidly frozen in a mixture of acetone and dry-ice, and stored in a −70°C freezer.

For the histochemical demonstration of monoamine oxidase (MAO) and acetylcholinesterase (AChE) activities, the fresh frozen sections at 8–12 μ thick were made in a −20°C cryostat with a sliding microtome. Serial cryostat sections were mounted on clear slides and were dried for 20 minutes at room temperature. For the demonstration of MAO activity, the tryptamine-tetrazolium method (13) was used. The substrate mixture solution contained 25 mg tryptamine hydrochloride, 5 mg nitro blue tetrazolium, 4 mg sodium sulfate, 5 ml of 0.1 M phosphate buffer (pH 7.4) and 15 ml of distilled water. Sections were incubated in this solution at 37°C for 30 minutes. For the demonstration of AChE, acetyl thiocholine method (14) was employed. Prior to being placed in the incubation solution at 37°C for 2 hours, the slides were kept at room temperature for 30 minutes in the preincubation solution containing 20 per cent solution of sodium sulfate and 10⁻⁶ M diisopropylfluorophosphate.

For quantitative analysis of MAO, the manometric method as described by Creasey (15) was performed, using 10⁻² M of tyramine hydrochloride, tryptamine hydrochloride, l-noradrenaline hydrochloride, serotonin creatinine sulfate and isoprenaline hydrochloride as substrate. The glands from group V and VI were minced and homogenized with a glass homogenizer in 0.25 M of cold sucrose, and then were centrifuged at 1,000 x g for 10 minutes and the supernatant was used as an enzyme source. The gas phase was air atmosphere and the reaction temperature was 37.5°C. The substrate was tipped into main compartment after 10 minutes of temperature equilibrium. After tipping, the readings were taken at 15 minutes intervals for 45 minutes. The enzyme activity was expressed as μl of O₂ consumed per hour per gram of the tissue.

RESULTS

I. General Observations

Groups treated with ISO (Group I and VI)

Five to 10 minutes after the injection of ISO in dose of 5 mg/kg, there was so much saliva in the mouth that it flowed out and moistened the lower lip and chaps. About 30 minutes after the administration, the rats had tachypnea and lacrimation and general malaise, and prostrated on the floor. Rats continued to be in this condition for about 2 hours. Within about 5 hours, however, they appeared to be almost fully recovered from the effects of ISO. After the 5th day of the administration, the rats showed baldness in
Fig. 1. Comparative changes of body and submaxillary gland weight of rat following administration of ISO, propranolol, ISO and propranolol, and ISO and nialamide during 14 days. Means±s.e.m (n=4).

Fig. 2. Changes of rat body and submaxillary gland weight after administration of ISO (80 mg/kg) during 7 days. Means±s.e.m (n=6).
the submaxillary region and the salivary glands were palpable. There was a slight decrease in body weight and a remarkable increase in the weight of the submaxillary glands. After the 7th day of the administration, the increase in weight of the submaxillary glands became about twice that of control. After the 14th day of the administration, the submaxillary glands grew heavier as shown in Fig. 1. After the injections of 80 mg/kg of ISO during the 7 days, the weight of the submaxillary glands clearly increased. This increase was about 5 per cent higher than that on the 7th day of the administration of 5 mg/kg of ISO (Fig. 2).

**Group treated with propranolol (Group II)**

The rats succumbed to an attack of diarrhea and were significantly decreased in body weight, and the general conditions became weak. Moreover, the weight of the submaxillary gland in both 7th and 14th day groups decreased about 24 and 20 per cent as compared with the control groups respectively (Fig. 1).

**ISO group combined with propranolol (Group III)**

General reactions were almost the same as those in ISO single administered group. However, the weight of the submaxillary gland remarkably decreased when compared with ISO single administered group on both 7th and 14th day in experimental stage (Fig. 1).

**ISO group combined with nialamide (Group IV)**

General conditions were rather similar to ISO single administered group. The rats showed a sign of dyspnea and prostrated on the floor after 30 minutes of the injection, and continued to be in this condition for about 2 hours. The submaxillary glands evidently increased in weight and size as the ISO single administration (Fig. 1).

**II. Histological Observations**

The acini in normal submaxillary gland were composed of a certain number of pyramidal cells with nuclei presented in their basal aspects. In the intercalated ducts, low

![Fig. 3. Normal submaxillary gland. Hematoxylin eosin. Numerous granular tubules and striated ducts are present. ×150](image-url)
FIG. 4. After the administration of small doses of ISO. Hematoxylin eosin. Striated ducts and granular tubules are decreased in size. $\times 150$

FIG. 5. ISO treated (1 week administration 5 mg/kg). Hematoxylin eosin. Acini showed slight enlargement of cell size. $\times 150$

FIG. 6. ISO treated (1 week administration 80 mg/kg). Hematoxylin eosin. Moderate enlarged acini are present. Striated ducts are flattened. $\times 150$
columnar epithelia were seen. The granular tubules were composed of many vacuoles and were filled with fine eosinophilic granules instead of the striations in their basal portions. The striated ducts frequently exhibited high columnar cells with well defined lumens, and usually parallel basal striations (Fig. 3). After ISO administration, the acinar cells of the submaxillary gland showed progressive increase in size. The acinar nuclei were also somewhat larger than those of control. The degree of enlargement of the acini increased as the period of the injection was prolonged until 14th day so far studied (Figs. 4–7). Mitotic figures of the enlarged acini were in some part visible (Figs. 8 and 9). Striated ducts and granular tubules of the submaxillary gland showed no sign of increase in size.

After the administration of high dose of ISO, striated ducts were flattened (Fig. 6) and, granular tubules were less obvious and became finally indistinguishable (Figs. 6 and 7).

Fig. 7. After the administration of high doses of ISO. Hematoxylin eosin. Granular tubules and striated ducts are not remarkable. ×150

Fig. 8. Higher magnification of Fig. 7. Hematoxylin eosin. Mitotic figures in the enlarged acini are seen. ×300

Fig. 9. Higher magnification of Fig. 7. Hematoxylin eosin. ×600
Fig. 10. Propranolol treated (1 week administration). Hematoxylin eosin. Acini are rather decreased in size. ×150

Fig. 11. ISO and propranolol treated (1 week administration). Hematoxylin eosin. Acini are decreased in size. ×150

Fig. 12. ISO and nialamide treated (2 weeks administration). Hematoxylin eosin. Acini are increased in cell size. ×150
FIG. 13. Normal submaxillary gland. MAO. Weakly active throughout the cytoplasm and moderately reactive in the basal part of the ducts. $\times 150$

FIG. 14. Propranolol treated (1 week administration). MAO. Slightly reactive in the acini. $\times 150$

FIG. 15. ISO and propranolol treated (1 week administration). MAO. Well defined ducts showed moderate enzymatic activity. $\times 150$

FIG. 16. Upper part: Normal submaxillary gland. Lower part: ISO treated submaxillary gland. MAO. More increased staining in the acini after 14 days administered ISO. $\times 150$
FIG. 17. Normal submaxillary gland. AChE. Intense AChE staining around the acini. \( \times 150 \)

FIG. 18. Normal submaxillary gland. AChE. Intense AChE staining around the acini. \( \times 150 \)

FIG. 19. Normal submaxillary gland. AChE. An intense activity in large nerve trunks and in nerve fibers of large excretory ducts. \( \times 150 \)
FIG. 20. ISO treated (2 weeks administration). AChE. Scanty AChE staining around the acini. ×150

FIG. 21. Propranolol treated (2 weeks administration). AChE. Note AChE staining around the acini. ×150

FIG. 22. ISO and propranolol treated (2 weeks administration). AChE. Marked AChE staining around the acini. ×150
In the enlarged submaxillary gland induced by low and high doses of ISO, however, no signs of inflammation with infiltration were observed. In propranolol administered group, the acini of the submaxillary gland showed atrophic figures and reduction in size. Striated ducts and granular tubules were observed to undergo a normal development of their shapes (Fig. 10). The acini of the submaxillary gland in the group of ISO and propranolol were smaller than those in ISO treated group. There were no clear alterations in striated ducts and granular tubules in ISO and propranolol group (Fig. 11). In ISO and nialamide treated group, the acini, striated ducts and granular tubules in the gland were almost similar to those of ISO treated glands in histological pattern (Fig. 12).

III. Histochemical Observations

**Monoamine oxidase:** The acini in normal submaxillary glands exhibited a faint MAO activity with homogenous staining. All the ductal cells exhibited a moderate activity in the basal parts (Fig. 13). After the ISO administration, the acinar cells with progressive hypertrophy showed a more increasing activity for MAO in the cytoplasm than that of

![Fig. 23. Effect of ISO treatment on MAO activity of rat submaxillary gland. Tyramine as substrate.](image)

![Fig. 24. Effect of ISO treatment on MAO activity of rat submaxillary gland. Tryptamine as substrate.](image)
control (Fig. 16). In the submaxillary gland treated with propranolol, MAO activity was the same in the acinar cells as in control (Fig. 14). The gland treated with ISO and propranolol exhibited somewhat a similar stainability to that of control (Fig. 15). After ISO combined with nialamide, the submaxillary glands showed no MAO activity.

Acetylcholinesterase: In normal submaxillary glands, the enzyme activity was confined to the fine nerve fibers surrounding the acini like network patterns (Fig. 17) and to nerve fibers of large excretory ducts (Fig. 19). Large nerve trunks showed an intense stainability (Figs. 18 and 19). The activity of AChE in the gland treated with ISO showed no clear alteration. The activity of the enzyme in surrounding acini was rather decreased in the gland treated with ISO (Fig. 20). The enzyme activity was intense around the acini in the propranolol treated glands (Fig. 21). In ISO and propranolol administered group, the AChE activity showed a moderate stainability in surrounding acini (Fig. 22). In ISO and nialamide administered group, the pattern of AChE-staining in the submaxillary gland was almost similar to that of ISO administered group.

IV. Biochemical Observations

Since MAO activity of ISO treated group showed a marked changes histochemically, a quantitative analysis was made on substrate specificity of this enzyme by means of

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<th>Time (min)</th>
<th>Control submaxillary gland</th>
<th>ISO treated submaxillary gland</th>
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<tr>
<td>15</td>
<td>10</td>
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the manometric procedure. Tyramine, tryptamine, l-noradrenaline, serotonin and isoprenaline were used as substrate. These experimental results were shown in Figs. 23 to 27. When the above mentioned amines were used as substrate, MAO activities of ISO administered submaxillary glands were found to have a higher degree of O₂ consumption than that of control. These increase in the MAO activities were about 7 to 10 per cent higher at 15 minutes after incubation with each substrate. These results were in agreement with the histochemical observations after the administration of ISO.

With these amines as the substrates, the oxidation rates by the control submaxillary gland were in the following order, tyramine, tryptamine, noradrenaline, serotonin and isoprenaline. The order of the oxidation rates by the ISO treated submaxillary gland was as follows, tryptamine, tyramine, noradrenaline, isoprenaline and serotonin.

**Fig. 27.** Effect of ISO treatment on MAO activity of rat submaxillary gland. Isoprenaline as substrate.

**DISCUSSION**

Since the original observation of the enlargement induced by ISO in the salivary gland by Selye et al. (2), there have been some reports (3–11) concerning the experimental progressive hypertrophy of the salivary gland.

In the present experiments, histological findings of the submaxillary gland treated with ISO showed an intense degree of the enlargement of the acini. It has been reported that the normal matured salivary glands hardly showed mitotic figures (16). It was observed that there were a slight increase in the mitotic changes of the enlarged acini during the treatment of ISO. The duct system including the granular tubules, however, showed a gradual decrease in size probably due to the compression by the enlarged acinar cells. Furthermore, it was difficult to discriminate the granular tubules after a large dose of ISO was repeated.

It has been reported that the submaxillary gland of rats has both α- and β-receptors for catecholamine in the salivary secretion (17). Since propranolol inhibited the response of the gland weight to ISO, it was suggested that the stimulation of β-receptor in sub-
maxillary glands corresponded to the increase in gland weight.

MAO activity in the gland was not affected by treatment with ISO plus propranolol, but it was completely abolished by the combined dose with nialamide during the administration of ISO. The submaxillary gland weight, however, increased obviously. There seemed to be no connection between increase in MAO activity and that in submaxillary gland weight induced by ISO.

The autonomic innervation of the salivary glands has long been a controversial question in the salivary secretion from the physiological point of view. It is believed that the glandular cells have double innervation, and acini of the submaxillary gland have a parasympathetic innervation (18). Histochemically, MAO activity shows the presence of adrenergic neurons (19, 20).

In the submaxillary gland, the enzyme was mainly localized in the acini and ducts. After the ligation of the excretory ducts, an intense reduction of MAO activity has been observed in the atrophied acini and ducts (21). In the present experiments, there was an increased activity of MAO in the enlarged acini after treatment with ISO. It seems of interest to note that MAO activity has some relations with the atrophy and enlargement of the glands.

Recently, MAO activity in the denervated submaxillary glands was demonstrated by some authors (22, 23). Different results were obtained using different type of substrates. In the present investigation, MAO activities of the ISO treated submaxillary glands with five substrates were 7 to 10 per cent higher than that of control.

There are some reports (24–26) on the histochemical distribution of AChE in the normal salivary gland. The localization of AChE activity was demonstrated in the ducts, arteries and along the periphery of the acini. The functional role of AChE in the salivary gland is not exactly understood. However, it is discussed that AChE activity in the surrounding acini may be related to the passage of substances through the wall of the acinar cell and the secretion or the absorption of substances in the duct cells (24–26). In the present study AChE stainability surrounding the acini was intense with propranolol, while it was scanty with ISO, although Seifert (11) has shown that the activity of AChE in the ISO treated submaxillary gland was stronger than that of control.

SUMMARY

The histological, histochemical and biochemical changes of the rat submaxillary gland were investigated after the administration of ISO, propranolol and in combination of ISO and propranolol, and ISO and nialamide.

After the daily administration of ISO for 1 and 2 weeks, the submaxillary gland showed an increase in weight and in cell size enlargement especially of acini. Histochemically MAO activity increased in the cytoplasm of acini. AChE activity decreased in the surrounding of the acini.

After the administration of propranolol once daily for 1 and 2 weeks, the submaxillary gland showed a decrease in weight and an atrophic figure. Histochemical MAO
stainability of the acini was nearly the same as that of control. AChE activity was slightly increased in the surrounding acini.

After the administration of both ISO and propranolol once daily for 1 and 2 weeks, the increasing effect of ISO on weight in the submaxillary gland was completely inhibited by propranolol. The activity of MAO and the distribution patterns of AChE in this gland were almost the same as those in the propranolol treated gland.

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