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INCREASED MONOAMINE OXIDASE ACTIVITY IN ISOPROTERENOL-STIMULATED SUBMAXILLARY GLANDS

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Abstract—Prolonged isoproterenol administration increases the monoamine oxidase (MAO) but not the cytochrome oxidase activity of rat salivary glands. This increase in MAO can be blocked by prior administration of propranolol or actinomycin D, but is not diminished by ganglionectomy or duct ligation. Three hours after isoproterenol an increase in MAO activity is detectable, but is not significantly inhibited by propranolol. No increase in cardiac MAO concentration was observed. It is suggested that the increased MAO activity may be related to increased enzyme protein in the stimulated secretory cells.

ISOPROTERENOL has been shown to cause a marked hypertrophy and hyperplasia of rat salivary glands.[1, 2] This is accompanied by an increase in the rate of RNA and DNA synthesis.[3, 4] Histochemical studies have indicated that there is an increased activity of several enzymes in the salivary gland, including succinodehydrogenase, acid phosphatase and monoamine oxidase (MAO), after isoproterenol administration.[5] However, no quantitative biochemical data are available on the increase in MAO activity nor have studies on the mechanism of this increase been reported. Previous work has shown that sympathetic denervation of the rat submaxillary gland results in a decrease in MAO activity, indicating that a portion of the MAO may be intraneuronal or at least neurally dependent.[6] However, extirpation of the superior cervical ganglion did not prevent the isoproterenol-induced increase in gland weight.[7]

In this investigation the relationship of isoproterenol-induced hypertropy to changes in MAO activity, and the effect of sympathetic denervation and duct ligation on the magnitude of the isoproterenol effects were examined. Our results demonstrate a marked increase in the concentration and amount of MAO in the isoproterenol-treated rat submaxillary gland independent of innervation or the functional integrity of the ducts.

METHODS

Male Sprague–Dawley rats weighing 125–150 g were given Purina laboratory chow and water *ad libitum*. Except where noted, animals received either 7·5 mg/kg of isoproterenol hydrochloride (Sigma Chemical Corp., St. Louis, Mo.) or saline s.c. at 8 a.m. and 5 p.m. for 4 consecutive days and were killed about 10 a.m. on the fifth day. Submaxillary duct ligature or superior cervical ganglionectomy or both were performed on the right side 7 or 8 days before beginning the isoproterenol or saline

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treatment. The left gland was used as the control in these experiments. Rats were killed by cervical dislocation; and heart and the right and left submaxillary glands were rapidly removed and chilled on cracked ice. Thirty to 60 mg of each tissue was homogenized in 50 or 100 vol. of 0·25 M sucrose in a glass homogenizer. Aliquots corresponding to 0·5 or 1·0 mg tissue were used to determind MAO activity,[8] protein[9] and cytochrome oxidase.[10] One MAO unit = 1 mμmole/hr at 37°. One cytochrome oxidase unit = amount of enzyme resulting in a change in the logarithm of the ferrocytochrome C concentration of 1·0 per min at room temperature. Actinomycin D. was kindly furnished by Dr. Robert R. Engle, National Cancer Institute, Bethesda, Md. Statistical significance was assessed by using either an analysis of variance with Tukey's procedure or a *t*-test.[11]

RESULTS

Effect of isoproterenol on salivary gland MAO. After the administration of isoproterenol for 4 days, there was a doubling in total MAO activity in the combined rat submaxillary and parotid glands and a 17 per cent increase in MAO concentration (Table 1). An increase in concentration of MAO activity per mg protein was observed

TABLE 1. INHIBITION OF ISOPROTERENOL-INDUCED SALIVARY MAO ACTIVITY BY PROPRANOLOL*

Treatment	Wt. salivary glands	MAO activity (mμmoles/hr)		
		Per mg wt.	Per mg protein	Per glands
Control	890 ± 23	4·07 ± 0·20	26·5 ± 0·8	3624 ± 207
Isoproterenol	1699 ± 37†	4·75 ± 0·15‡	26·2 ± 0·8	8070 ± 272†
Propranolol + isoproterenol	898 ± 44	4·34 ± 0·15	27·2 ± 1·2	3867 ± 166

* Treated animals received either 7·5 mg/kg of isoproterenol HCl s.c. or propranolol, 20 mg/kg i.p. 5 min before 7·5 mg/kg of isoproterenol HCl twice daily for 4 days. Animals were killed on the fifth day and the MAO activity of the combined submaxillary and parotid glands was determined. Results of 8 determinations in each group are expressed as the mean ± S.E.M.

† $P < 0.01$.

‡ $P < 0.05$.

in the submaxillary gland (see Figs. 2–4), but not when the submaxillary and parotid were examined together (Table 1). Pretreatment with the β blocking agent, propranolol, prevented the isoproterenol-induced increase in both gland weight and MAO activtiy. A small but significant increase in MAO activity per mg protein was apparent in the submaxillary gland as early as 3 hr after a single dose of isoproterenol (Table 2), at which time the acute cardiovascular effects of isoproterenol had disappeared.[12, 13] This increase in MAO activity per mg protein was not significantly diminished by prior administration of propranolol.

Since MAO is associated with the mitochondria in the liver,[14] cytochrome oxidase, an enzyme confined to this subcellular fraction, was determined in isoproterenol-treated and untreated submaxillary glands. Unlike the changes in MAO activity, the activity of cytochrome oxidase was not significantly increased (Fig. 1). This effect is

probably not due to a direct inhibitory effect of isoproterenol on cytochrome oxidase, since no isoproterenol or its 3-methoxy derivative is present in this tissue 16 hr after adminstration.[12]

TABLE 2. ACUTE EFFECT OF ISOPROTERENOL ON SALIVARY GLAND MAO ACTIVITY*

Treatment	Salivary wet wt.	MAO activity (mμmoles/hr) Per mg wt.	Per mg protein	Per glands
Control	823 ± 33	2·51 ± 0·28	33·5 ± 3·4	2021 ± 218
Isoproterenol	881 ± 36	3·01 ± 0·25	41·2 ± 2·8†	2644 ± 237†
Propranolol + isoproterenol	826 ± 20	3·22 ± 0·18	39·4 ± 2·3	2659 ± 154†

* Treated animals received either 7·5 mg/kg of isoproterenol HCl alone or 5 min after propranolol, 20 mg/kg i.p. The animals were killed 3 hr after isoproterenol and the combined submaxillary and parotid gland MAO was determined. The results of 8 determinations in each group are expressed as the mean ± S.E.M.
† $P < 0·05$.

To determine whether the increased MAO activity of the isoproterenol-treated salivary gland is due to new protein synthesis, secondary to an increase in the rate of formation of messenger RNA, the effect of actinomycin D was examined. This compound markedly reduced both the magnitude of the increase in gland size and MAO activity induced by isoproterenol (Fig. 1).

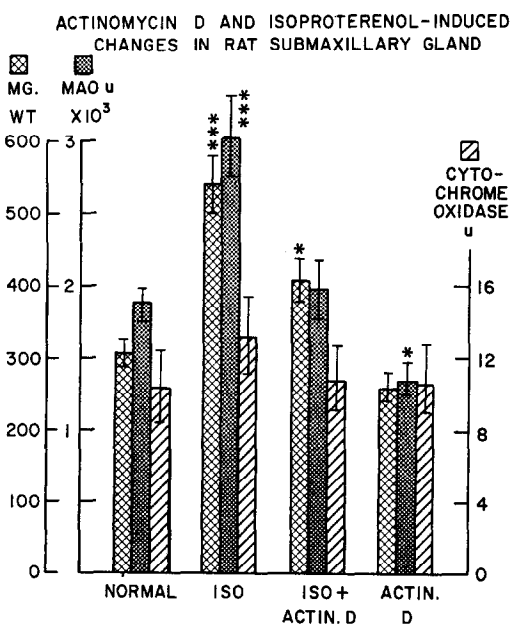

FIG. 1. Inhibition of isoproterenol-induced changes of rat submaxillary glands by actinomycin D. Treated animals received either isoproterenol HCl, 7·5 mg/kg s.c., or actinomycin D, 150 μg/kg i.p. or actinomycin D 20 min before isoproterenol HCl at zero time, 16 hr and 24 hr. Animals were killed 40 hr after the first injection and the weight, cytochrome oxidase and monoamine oxidase activities were determined and expressed on a per gland basis. The mean and S.E.M. of 5–8 observations of each parameter are reported. *$P < 0·05$; ***$P < 0·001$.

In order to assess the possible presence of an enzyme activator in the isoproterenol-treated salivary gland or of an inhibitor in the untreated gland, homogenates of normal and stimulated salivary glands were combined and MAO activity was measured. Enzyme activity of the combined homogenates was not different from that expected from the activity of each homogenate alone.

Since isoproterenol has been reported to induce cardiac hypertrophy, which can be blocked by prior administration of an MAO inhibitor,[13] the MAO activity of this organ was also determined. Treatment with isoproterenol for 4 days caused an increase in both heart weight and total MAO activity. Unlike the salivary gland, however, no increase in MAO concentration was observed in the heart (Table 3). These changes are similar to those observed in the salivary gland after only one (Table 2) or three (Fig. 1) doses of isoproterenol.

TABLE 3. CARDIAC MAO ACTIVITY AFTER ISOPROTERENOL-INDUCED HYPERTROPHY*

Treatment	Heart wt.	MAO activity (mμmoles/hr)		
		Per mg wt.	Per mg protein	Per heart
Control	549 ± 16	3·50 ± 0·26	22·1 ± 1·9	1929 ± 155
Isoproterenol	798 ± 34†	3·67 ± 0·35	26·4 ± 2·7	2903 ± 288†

* Treated animals received 7·5 mg/kg of isoproterenol HCl s.c. twice daily for 4 days. Animals were killed on the fifth day and the cardiac MAO was determined. The results of 8 animals in each group are expressed as the mean ± S.E.M.
† $P < 0.01$.

Effect of sympathectomy and duct ligation. In order to determine if the increase in MAO activity after isoproterenol administration was neural, extraneural or both, rats with denervated submaxillary glands were used. In confirmation of previous

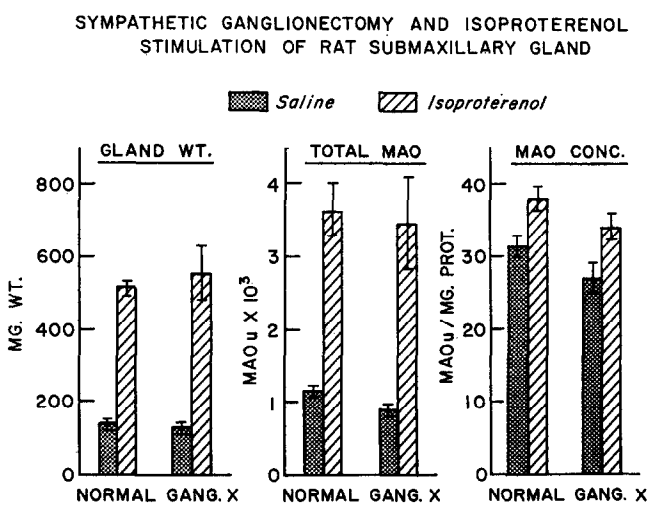

FIG. 2. Effect of sympathetic ganglionectomy on isoproterenol-induced changes in rat submaxillary glands. The right superior cervical ganglion was removed 7 days prior to beginning treatment with isoproterenol HCl, 7·5 mg/kg, or 0·2 cc saline s.c. twice daily for 4 days. Animals were killed on the 12th day after surgery and the right (gang. X) and left normal) submaxillary glands were removed. Each column and bracket represents the mean ± S.E.M. of 8 determinations.

findings,[6] this procedure decreases the MAO activity ($P < 0.05$) of untreated salivary glands whether MAO activity is expressed per milligram of protein or whole gland (Fig. 2). In the absence of sympathetic innervation, the stimulatory effect of isoproterenol on gland weight, total MAO or MAO concentration was as marked as in the normal innervated glands (Fig. 2).

After duct ligation, submaxillary gland MAO activity was reduced ($P < 0.01$), as previously reported.[15] This prompted a study on the effect of isoproterenol treatment on the MAO content of submaxillary glands after duct ligation. Even in the glands with ligated ducts there is a marked increase in gland weight, total MAO and MAO concentration (Fig. 3). The relative increase in gland weight or total MAO concentration is similar to that observed in normal glands, although the absolute value is less.

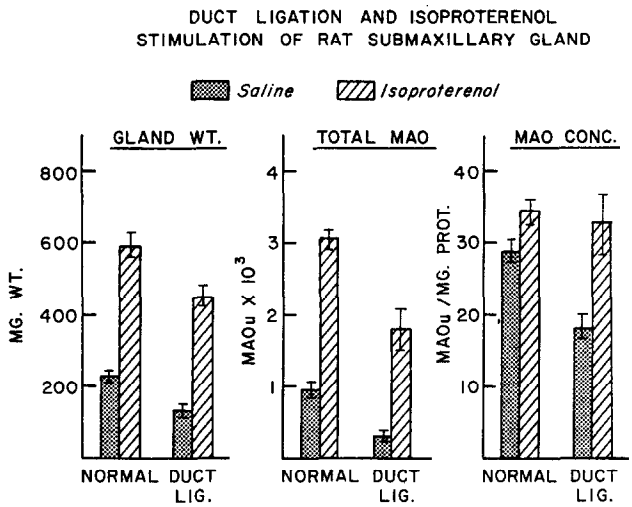

FIG. 3. Effect of duct ligation and isoproterenol-induced changes in rat submaxillary glands. The right submaxillary gland duct was ligated 7 days prior to beginning treatment with isoproterenol HCl, 7·5 mg/kg, or 0·2 cc saline s.c. twice daily for 4 days. Animals were killed on the 12th day and the right (duct lig.) or left (normal) submaxillary glands were removed. Each column and bracket represents the mean ± S.E.M. of 7 determinations.

The effect of combined duct ligation and sympathetic denervation on gland weight, total MAO and MAO concentration was found to be more marked than that of either procedure alone ($P < 0.05$). However, the isoproterenol-induced increase in submaxillary gland weight, total MAO and MAO concentration was essentially the same as that of the intact normal gland (Fig. 4).

DISCUSSION

Histochemical studies have demonstrated an increase in MAO, acid phosphatase and succinodehydrogenase activities after isoproterenol administration.[5] Since enlargement of mitochondria and parenchymal hyperplasia are pronounced after isoproterenol stimulation, it is possible that the changes in MAO and succinodehydrogenase are merely a reflection of increased mitochondrial mass. In the studies described here, an increase in MAO activity by direct biochemical measurement was also found. However, there was no change in the mitochondrial matrix enzyme, cytochrome oxidase, after isoproterenol administration. By the use of an inhibitor

of RNA sythesis, our studies indicate that some of the increased MAO activity is probably a consequence of increased synthesis of MAO relative to its destruction.

The slight increase in MAO activity within 3 hr of isoproterenol administration, at a time when the increased RNA and DNA synthesis rates have not yet occurred,[4] may imply an initial stimulus to MAO synthesis more remote from the genetic transcription process. Propranolol, which has previously been shown to block completely the isoproterenol stimulation of salivary gland growth,[16] did not completely reverse the small increase in MAO acivity in this experiment.

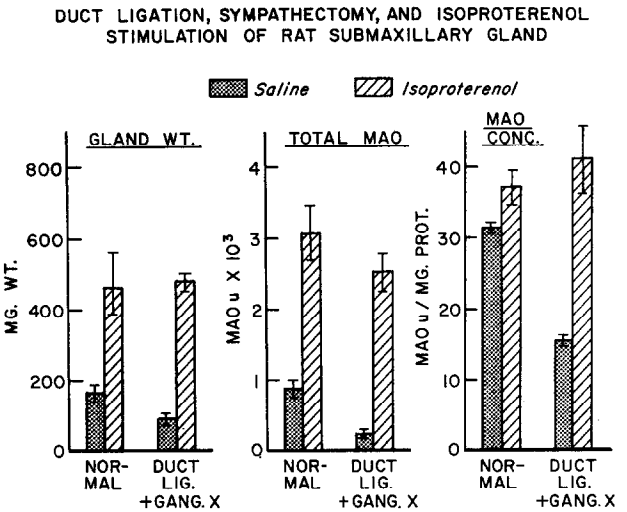

FIG. 4. Effect of sympathetic ganglionectomy and duct ligation on isoproterenol-induced changes in rat submaxillary glands. The right submaxillary gland duct was ligated and superior cervical ganglion was removed 8 days prior to starting treatment with isoproterenol HCl, 7·5 mg/kg, or 0·2 cc saline s.c. twice daily for 4 days. Animals were killed on the 13th day and the right (duct lig. + gang. X) or left (normal) submaxillary glands were removed. Each column and bracket represents the mean ± S.E.M. of 6 determinations.

These findings indicate either that isoproterenol may affect mitochondrial enzymes unequally or, more likely, that the majority of the isoproterenol-stimulated MAO activity is not mitochondrial. A major fraction of the MAO in this tissue appears to have a microsomal location.[17]

Although a small portion of submaxillary MAO may be located within sympathetic nerve terminals, sympathetic denervation does not alter the ability of isoproterenol to increase the salivary gland weight.[7] The isoproterenol induced increase in MAO activity is also unaltered by sympathetic denervation, which indicates that the isoproterenol-induced enzyme activity is neither located within nerve terminals nor neurally dependent.

Isoproterenol produces a marked increase in very viscid saliva production,[2] but the suppression of secretion by duct ligation does not abolish the isoproterenol stimulatory effect. Duct ligation does diminish the absolute but not the relative weight and MAO activity concentrations achieved after isoproterenol administration. The altered resting gland weights and MAO levels seen after combined superior cervical ganglionectomy and duct ligation are most easily explained by a summation of the changes observed when either procedure is used alone.

Increased MAO activity develops after stimulation of secretion in salivary glands by either pilocarpine[18] or isoproterenol. Both of these compounds produce an increase in amount of salivary gland endoplasmic reticulum within a few hours of administration.[19, 20] The microsomal localization of a portion of salivary gland MAO and the association of this subcellular fraction with protein synthesis suggest that the increased MAO activity observed in these studies is a reflection of the increased synthetic activity in the stimulated secretory cells.

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