Changes in Peroxidase Activity in Salivary Gland After Administration of Isoproterenol* (34031)

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(Introduced by Sidney Weinhouse)

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Isoproterenol, a sympathomimetic amine, stimulates cell proliferation in the salivary glands of rodents (1, 2). A single dose of isoproterenol causes a burst of DNA synthesis which reaches a peak in mice after approximately 28 hr and is followed by a wave of mitosis (2). Thus, the isoproterenol-stimulated salivary gland provides a system which has some unique features for investigating metabolic events relative to cell proliferation.

Previous studies have demonstrated high peroxidase activity in proliferating tissue (3, 4). Since peroxidase normally is present in the salivary glands of a number of species (5–8), it was of interest to investigate peroxidase activity in the salivary gland after its stimulation with isoproterenol.

Materials and Methods. Fels A male mice, approximately 4 months old and weighing 30 g were used. Isoproterenol was injected intraperitoneally, 1.0 μmole/g body wt. of a freshly prepared solution of chromatographically pure isoproterenol HCl [1-(3, 4, -dihydroxphenyl)-2-isopropylaminoethanol hydrochloride, from Winthrop Laboratories, New York, New York]. Actinomycin D (1.0 μg/g body wt.) was purchased from Mann Research Laboratories, New York, New York.

Animals were sacrificed at the times indicated and the salivary glands removed and frozen at —20° for no longer than 48 hr prior to analysis.

Salivary glands from at least two animals were pooled, minced, and washed with 10 vol of cold 0.01 *M* phosphate buffer, pH 7.4. A 20% homogenate was prepared in 0.01 *M* phosphate buffer, pH 7.4, using a Dounce homogenizer with loose and tight pestle. The homogenates were filtered through a double layer of cheesecloth and centrifuged at 45,000*g* for 1 hr using an IEC B-20 centrifuge. The pellet was resuspended in the phosphate buffer and the supernatant fluid was discarded unless otherwise indicated.

Protein determinations were carried out using the Folin reagent by a slight modification of the procedure of Heidelberger and MacPherson (9, 10). Peroxidase activity was determined by following the rate of oxidation of reduced 2, 3′, 6, trichloroindophenol essentially as described previously (11), using a Coleman Jr. spectrophotometer. A unit of peroxidase activity is defined as that amount of enzyme which causes a Δ log I per minute of 1.0 under the specified conditions of the assay. Hemin was measured as the pyridine hemochromogen as described by Neufeld *et al.* (4), using a Cary Model 14 recording spectrophotometer.

Results. Peroxidase activity of salivary glands from several species has been reported (8); however, to our knowledge no information was available for the mouse. Results shown in Table I indicate that the mouse salivary gland contains peroxidase activity. Seventy-three per cent of the peroxidase activity of the homogenate of the gland could be attributed to pseudoperoxidase activity based on hemin analyses. The remaining peroxidase activity sedimented as a pellet with particulate material and was interpreted to represent the true peroxidase activity of the salivary gland. These results are in agreement with previous findings which showed

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TABLE I. Peroxidase Activity of Mouse Salivary Gland.

	Total peroxidase (units/ml)	Pseudoperoxidase[a] (units/ml)
Homogenate	15.0	—
Pellet	4.8	0
Supernatant fluid	11.0	10.5

[a] The pellet was resuspended in buffer to the original volume of the homogenate. The resuspended pellet and supernatant fluid were analyzed for hemin, and pseudoperoxidase activity calculated assuming 1 unit of activity/2 μg hemin, based on earlier findings (4). The specific activity of the homogenate, expressed as units/mg protein, was 0.79; of the pellet, 0.87 and of the supernatant fluid, 0.55. In three separate experiments, 73–85% of the peroxidase activity of the homogenate was localized in the supernatant fluid.

that homogenates of a variety of animal tissues contained both peroxidase and pseudoperoxidase activity, the latter remaining in solution at relatively high centrifugal forces (4). All subsequent experiments deal only with true peroxidase activity.

Stimulation of the salivary gland by isopreterenol resulted in changes in peroxidase activity as shown in Fig. 1. An initial decrease was followed by an increase which reached a maximum approximately 32 hr after administration of isoproterenol. Peroxidase activity had returned to normal by 72 hr.

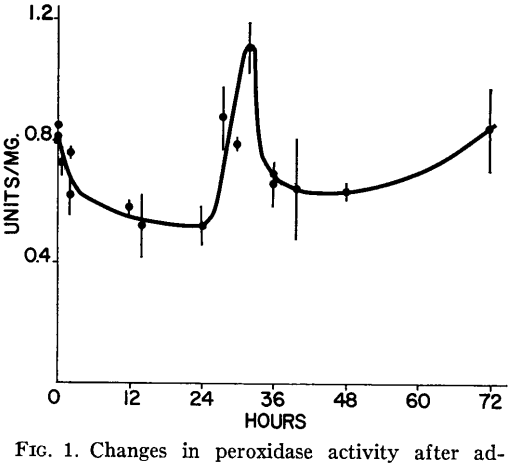

FIG. 1. Changes in peroxidase activity after administration of isoproterenol. Each point represents the average of results obtained from two different preparations for a given time after injection of isoproterenol. The vertical bars indicate the range in values obtained.

As shown in Table II, administration of actinomycin D prior to, or after treatment with isoproterenol, had no effect on the peroxidase activity of the salivary gland. Under the conditions of the experiment, stimulation of DNA synthesis by isoproterenol would have been blocked (12).

Since the peroxidase activity of various tissues can be increased by the addition of dichlorophenol to the assay system (11, 13) the effect of this compound on the peroxidase activity of the salivary gland prior to and after stimulation with isoproterenol was compared. In a similar manner, the effect of KI was investigated. The results obtained for the nonstimulated gland are summarized in Table III. Similar results were obtained with the stimulated gland. Both dichlorophenol and KI increased the peroxidase activity of the gland, the extent of the increase being dependent on the concentration of these compounds. When both dichlorophenol and KI were present in the assay mixture maximal rates were obtained only at lower concentrations of these compounds. At higher concentrations, the rates were less than those obtained with either compound alone.

TABLE II. Effect of Actinomycin D on Peroxidase Activity of Isoproterenol-Stimulated Salivary Gland.

	Units/mg
Control	0.81 ± 0.005
27 hr after IPR[a]	0.88 ± 0.105
Actinomycin D	
30 min before IPR	0.76 ± 0.005
12 hr after IPR	0.72 ± 0.130
20 hr after IPR	0.94 ± 0.005
24 hr after IPR	0.91 ± 0.011

[a] All animals given isoproterenol (IPR) were sacrificed 27 hr later. Animals received actinomycin D at the times indicated. Each value represents the average of results obtained from two different preparations. The standard deviation is included.

Discussion. Results from the present study show that peroxidase activity of the mouse salivary gland can be affected by the admin-

TABLE III. Effect of Dichlorophenol and KI on Peroxidase Activity of the Salivary Gland.

DCP (μmoles)	Activity (units/ml)	KI (μmoles)	Activity (units/ml) —DCP	Activity (units/ml) +DCP[a]
		0.0	13.5	21.9
0	13.5	0.2	14.7	40.5
15	21.9	1.0	18.5	—
30	21.9	2.0	27.6	30.0
45	12.3	6.0	31.3	—
		12.0	40.5	28.8

[a] Fifteen μmoles of 2,4 dichlorophenol (DCP) was added to the assay mixture containing the amount of KI as indicated; the specific activity in the absence of DCP and KI was 0.85.

istration of isoproterenol. An increase in activity, though small, occurs approximately 32 hr after the administration of isoproterenol; this is during the period of mitosis and approximately 5 hr after peak DNA synthesis. The initial decrease in peroxidase activity which is observed may be due to increased secretion of peroxidase from the gland.

In the present study the increase in peroxidase activity was not inhibited by administration of actinomycin D. These results are similar to those obtained for amylase (14) but in marked contrast to results obtained for thymidine kinase, whose increase is effectively inhibited by actinomycin D (14). Results from the present study suggest that in the mouse salivary gland, under conditions of cell proliferation, an increase in peroxidase activity does not represent synthesis of peroxidase from a newly supplied template, but results on the contrary from a stable template.

Peroxidase of the mouse salivary gland appears to be firmly bound and not easily solubilized. Attempts to solublize the enzyme using dimethysulfoxide were unsuccessful. These results are in marked contrast to those reported by Thomson and Morell (8) for peroxidase from salivary glands of many other species. These authors found 70–90% of the peroxidase activity present in a 200,000*g* supernatant fraction. The discrepancies may be due to some peculiar difference between species or to solubilization of the enzyme under their experimental conditions, although no precaution appears to have been taken to correct for pseudoperoxidase activity.

Dichlorophenol and potassium iodide were studied to determine if the various peroxidase samples would all respond in a similar manner, which they did. Of interest also, was the observation that KI, but no KCl, increased the rate of oxidation of the indophenol dye used in these peroxidase assays. Recently Hati *et al.* (15) reported that goat submaxillary gland contained a new type of peroxidase which was stimulated by KI. In view of the findings of Morrison, Allen, and coworkers (5–7) demonstrating for several species, the immunochemical identity between salivary gland peroxidase and lactoperoxidase from milk, it would appear that the peroxidase of Hati *et al.* (15) would in all likelihood be lactoperoxidase. Although it has been reported that iodide does not stimulate the oxidation of dianisidine by thyroid peroxidase (16), in the present studies, the activity of mouse salivary gland peroxidase and bovine lactoperoxidase (milk) were both increased by the addition of iodide to the assay. Under the conditions of our assay 5 μmoles KI increased the activity of bovine lactoperoxidase from 137 to 814 units/mg protein.

Summary. Stimulation of mouse salivary gland by isoproterenol resulted in an initial decrease in peroxidase activity, followed by an increase with subsequent return to normal values. Actinomycin D did not block the increase in peroxidase activity observed.

Both dichlorophenol and KI increased the peroxidase activity of the nonstimulated and of the isoproterenol-stimulated salivary gland.

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α-Methyl-1-Adamantane-Methylamine Hydrochloride: Inhibition of Rous and Esh Sarcoma Viruses in Cell Culture* (34032)

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Our work and that of others, employing 1-adamantanamine hydrochloride (amantadine HCl, Symmetrel) to inhibit Bryan strain Rous sarcoma (RSV) and Esh sarcoma viruses (ESV) (1, 2) in cell culture led to our interest in α-methyl-1-admantanemethylamine hydrochloride (methyl amantadine HCl, MA). Studies indicate that MA was more effective in inhibiting influenza virus *in vitro* than was amantadine HCl (3). MA also inhibited the growth of other myxoviruses *in vitro,* especially rubella and rubeola, as well as respiratory syncytial, parainfluenza 2 and 3.

Materials and Methods. Bioassay of the two avian oncogenic viruses was similar to that of Rubin (4), except that chick embryo fibroblasts that had been frozen in a dimethyl sulfoxide-medium mixture and stored in ampules in liquid nitrogen were used for focus production (5). The growth medium contained, in addition to the original ingredients, Eagle's basal medium and calf serum (rather than chicken serum that might contain antibodies to avian leukosis viruses).

MA was added to the tubes of chick embryo cells, and the tubes were then placed in a water bath at 37° for 15 min. Immediately, the drug–cell suspension was pipetted into Falcon petri dishes (60 × 15 mm with 2-mm grids), and the various dilutions were added to the plates within 5 min. The next day, the cultures were overlaid with agar that contained MA and on day 4 postinoculation, feed medium (also containing MA) was added to all of the plates. After incubation in a CO_2 incubator (4%) and >85% relative humidity, the virus-induced foci were counted.

Results. Figure 1 shows the data obtained in graphic form. The mean concentration of MA to give 50% inhibition of virus with RSV was 7.4 μg/ml (range 6.0–9.7). For ESV, a mean concentration of 5.8 μg/ml (range 4.5–7.2) was necessary for 50% inhibition. The mean values for 50% inhibition using amantadine HCl was 18.4 μg/ml for RSV and 11.3 for ESV (1). Lower concentrations were also noted for the effect of MA with the myxoviruses (3).

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